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COMPARISON OF DATA OBTAINED BY HPLC AND MICROBIOLOGICAL DETERMINATION OF RIBOFLAVIN IN READY-TO-EAT FOODS

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Riboflavin content of ten kinds of preserved baby foods containing a variety of vegetables and milk, meat, liver or fish were determined by microbiological method and by HPLC. Homogenized food samples were heat treated by acid for microbiological assay. For HPLC analysis the acid treatment was followed by an enzymatic digestion and a sample clean-up on Sep-Pak columns. Then reversed phase separation was applied with UV detection. Microbiological determination was performed with *Lactobacillus casei* and by trimetric evaluation.

The correlation coefficient between the data obtained by microbiological and HPLC analysis was 0.987. However in a few cases HPLC showed higher riboflavin content than the microbiological assay. The possible reasons of this discrepancy is discussed.

The detection limit of riboflavin in the case of HPLC method is 30 ng cm^{-3} injected solution, and its accuracy and reproducibility is suitable for determination of natural riboflavin content in most of foods. The daily riboflavin intake can be determined by HPLC in a shorter time than by microbiological assay.

Keywords: riboflavin, ready-to-eat food, HPLC, microbiological assay

The microbiological riboflavin assay of A. O. A. C. (1980, 43.126–43.172) is very sensitive and selective but it needs too much time and manual work and in the presence of antimicrobiological substances (antibiotics, preservatives) it can not be used.

Numerous reversed phase HPLC methods have been elaborated for the determination of riboflavin content in foods or enriched foods, employing either UV (TOMA & TABEKHIA, 1979; ASHOOR et al., 1983, 1985; KAMMAN et al., 1988) or fluorescence detector (FELLMAN et al., 1982; AUGUSTIN, 1984; FINGLAS & FAULKS, 1984; MAURO & WETZEL, 1984; WEHLING & WETZEL, 1984; KNEIFEL, 1986; JOHNSSON & BRANZELL, 1987; DAWSON et al., 1988). Our aim was to elaborate a convenient method with UV detection after a sample clean-up and its application for complex food samples containing vegetables or fruits and meat, fish, milk or liver, while the earlier HPLC methods with UV detection could be used only for simple food samples.

1. Materials and methods

The riboflavin assay was performed with HPLC and the microbiological method on 10 kinds of heat-preserved ready-to-eat baby foods. The major components are shown in Table 1.

Table 1

Riboflavin concentrations measured in heat-preserved baby foods by HPLC and by Lactobacillus casei

Sample	Riboflavin (μg per 100 g) measured by			
	HPLC		L. casei	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
	n = 4		n = 5	
Diverse vegetables with fish mash	43	2.4	38	6.6
Apple-banana with chicken meat mash	54	2.9	43	2.9
Carrot with chicken meat mash	46	1.7	46	4.8
String beans with beef mash	34	1.5	49	7.9
Green peas with beef mash	62	2.4	56	4.7
Diverse vegetables with beef mash	61	4.9	65	11.0
Potatoe-tomato with fish mash	92	3.8	78	10.0
Pumpkin with milk	123	9.0	104	18.0
Diverse vegetables with pork liver mash	260	12.0	192	17.0
Potatoe-tomato with pork liver mash	270	6.3	240	24.0

1.1. Microbiological method

Bacto Riboflavin Assay Medium (Difco) and *Lactobacillus casei* (ATCC 7469) was used for the microbiological assay. The riboflavin content was determined by titrimetry after an incubation period (72 h, at 37 °C) according to A. O. A. C. (1980).

1.2. HPLC method

1.2.1. Reagents. Clara-diastase (Fluka) was suspended (2 g 100 cm^{-3}) in sodium acetate buffer (2.5 mol dm^{-3}, pH: 4.5). Papain (Reanal) was suspended in distilled water (5 g 100 cm^{-3}). Acetonitrile (Reanal) and methanol (Reanal) was refined by distillation.

Disposable reversed-phase C_{18} columns (500 mg) were obtained from BST (Hungary). Potassium phosphate buffer (0.01 mol dm^{-3}, pH: 7.0) : acetonitrile (89.5 : 10.5) was used as the mobile phase. It was filtered and degassed previously. The riboflavin stock solution contains 10 mg riboflavin (Fluka) in 100 cm^3 water.

1.2.2. Apparatus. Liquochrom HPLC system, model 2010 (made in Hungary) was used having an UV detector operating at 268 nm and 0.01 AUFS.

1.2.3. Chromatographic conditions. Chromatographic separation was achieved using a Nucleosil C_{18} column (250×4.6 mm id.) of 5 mm diameter

particle size and a guard column (50×4.6 mm) packed with Nucleosil C_{18}, particle size 10 μm. The columns were loaded with an aliquot of 50 mm^3 injected solution and they were used at 41 °C. The flow rate of the mobile phase was set at 1.0 cm^3 min^{-1}. At the end of the daily work the column was washed with water: acetonitrile 80 : 20 mixture to remove the water soluble components and then it was stored in an acetonitrile: water 80 : 20 mixture.

Quantification was based on peak height comparison with standards. The peak height was measured from the line connecting the start point and end point of the peak. The retention time of riboflavin was 20.5 min.

1.3. Procedure

1.3.1. Preparation of samples. Ahom ogenized food sample of approximate ly 5 g was suspended in 20 cm^3 of 0.1 mol dm^{-3} hydrochloric acid and then autoclaved at 103.5 kPa for 20 min. After cooling to room temperature the sample was filtered, the pH was adjusted to 6.8 and it was brought to volume with water. This solution after appropriate dilution was suitable for the microbiological assay. The undiluted solution had to be digested by 2 cm^3 of Clara-diastase suspension and 2 cm^3 of papain suspension at pH 4.5 for 20 h at 37 °C before the HPLC assay. Then the proteins were precipitated by adding 2 cm^3 of trichloroacetic acid (50 g 100 cm^{-3}) and heating for 10 min in boiling water bath. After cooling to room temperature and adjusting the volume the precipitate was removed by centrifugation and the supernatant was filtered through paper (Faltenfilter, Macherey–Nagel 615).

Concentration and further purification of the clear extracts was carried out using a short disposable column containing 500 mg octadecylsilane cartridge. The column was preconditioned by washing twice with methanol (2 cm^3) followed by double-distilled water (twice 2 cm^3). The sample solution (4–12 cm^3) was applied on a Sep-Pak column which was washed twice with 2 cm^3 of 0.01 mol dm^{-3} phosphate buffer (pH: 4.0) followed by 2 cm^3 of 0.01 mol dm^{-3} phosphate buffer (pH: 4.0) : methanol 95 : 5 mixture. Riboflavin was eluated with water : methanol 60 : 40 mixture and the volume of the collected solution was adjusted to 4 cm^3. Samples were protected from light during the whole process by covering tubes and flasks with aluminium foil.

2. Results

The chromatograms of the baby foods were evaluated on the base of the calibration curve obtained from the riboflavin working solutions in a concentration range from 0.05 μg cm^{-3} to 0.5 μg cm^{-3}. One food sample was

injected four times and the riboflavin data are shown in Table 1. In the blank solution the riboflavin peak was not detectable.

A statistical comparison between the results from 10 baby foods processed by the HPLC and the microbiological method gave a linear regression straight ($y = 1.26x - 9.87$) with a coefficient of 0.987. This value indicates a good correlation, although in few cases the results obtained by HPLC were higher than those obtained by the microbiological method (Fig. 1).

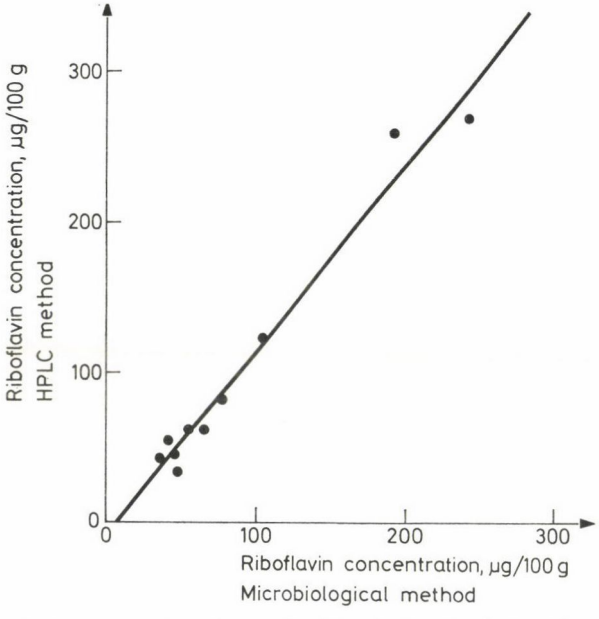

Fig. 1. Correlation between results determined in baby foods by the microbiologica method and by HPLC. $y = 1.26x - 9.87$; $r = 0.987$

The recovery of riboflavin was found 90 $\pm$ 7%, adding riboflavin to four samples before extraction in an amount similar to that originally present. The reproducibility expressed by VC was on the average 10.5% when the riboflavin content was determined in 4 food aliquots studied in parallel. Assaying the riboflavin concentration four times in the same sample solution the variation coefficient was 5.4% on the average. The linearity of the calibration curve was satisfactory having a linear regression coefficient of 0.998. The limit of detection was 30 ng cm^{-3} injected solution using signal-to-noise ratio of 3.

The microbiological results shown in Table 1 were obtained from five parallel assays.

The Fig. 2 shows the chromatogram of one of the baby foods. This sample was "string beans with beef mash".

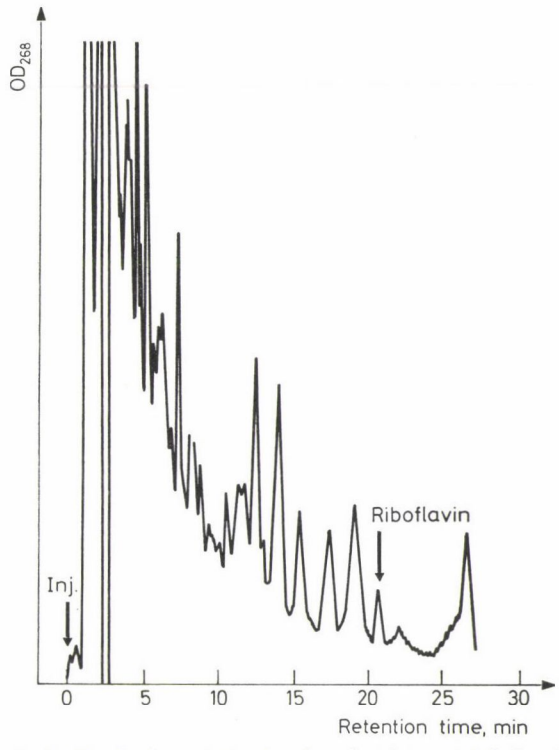

Fig. 2. Separation of riboflavin in a baby food string bean with beef mash (by HPLC) on a column packed with Nucleosil C_{18} parcticle size 5 μm, the mobile phase was 0.01 mol dm^{-3} potassium phosphate buffer (pH = 7.0) – acetonitrile 89.5 : 10.5 with UV detection at 268 nm

3. Discussion

Comparing the riboflavin values determined by the microbiological method and by the HPLC method with fluorescence detection JOHNSON & BRANZELL (1987) found a linear regression coefficient of 0.9996 in case of gruels, infant formulae, breakfast cereals and flours but in unenriched flours and flours with high rate of extraction the HPLC method gave much lower results (by about 25–50%) than the microbiological method. It was assumed that the different isometric riboflavin monophosphates had different sensitivities to enzymic cleavage.

When the HPLC method is used for the determination of riboflavin content of unenriched food the cleavage of the flavine monophosphate is necessary in the mixture after acid heat treatment. In the microbiological technique monophosphate is biologically active as nutrient for the Lactobacillus growth. For enzymatic digestion of FMN Clarase (Fluka) and Taka-diastase (Fluka) preparation were used in some food types previously and the Clarase in the

same concentration was found more efficient therefore Clarase was applied in the enzymatic digestion of baby foods. Supplementing the digestion with papain the chromatograms with UV photometric detection were clearer and more easily evaluable than without proteolytic treatment. The sample clean-up was performed similarly to the procedure published by FELLMAN and co-workers (1982). The mobile phase for the separation of riboflavin in baby food samples was a modification of the eluent used by KAMMAN and co-workers (1988) for the assay of thiamine and riboflavin in enriched cereals. The presence of an ion-pair reagent was not necessary in determination of riboflavin.

The higher riboflavin figures obtained in a few cases by the HPLC method comparing to the microbiological results were not due to the disturbing compounds from the enzymatic treatment because the enzymatic blank did not show a measurable riboflavin peak. Probably the reason of the difference was the absence of the complete separation of the biologically effective molecules. The purity of the riboflavin peak could be confirmed in some respect by determining the riboflavin concentration of the solution at an other wawelength, too, for example at 254 nm.

These results pointed out that the presented HPLC method can be used for the determination of riboflavin in raw and prepared foods for the estimation of riboflavin intake.

*

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TOTAL AND STARCH LIPIDS OF SOME WHEAT CULTIVARS GROWN IN HUNGARY

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Starch lipids of 15 wheat cultivars grown in Hungary have been studied. After removing free lipids from wheat whole meal samples with n-hexane and bound lipids with water saturated n-butanol starch lipids have been extracted in a water bath with a mixture of n-propanol – water (3 : 1, v/v). Starch lipids have been separated by column chromatography into non-polar, glyco- and phospholipids, then further lipid components were determined by thin-layer chromatography. It was found that one third of wheat lipids belong to starch lipids, and two thirds are non-starch lipids. Within the starch lipids the amount of polar lipids (93.2%), and within these the amount of phospholipids was considerable (90.3%). Relationships between baking quality parameters (amount of wet gluten content, spreading of wet gluten, Hagberg falling number, Valorigraph value, Valorigraph water absorption, loaf volume, form ratio of loaf) and lipid data were investigated with the aid of linear regression analysis. For the wheat samples investigated no relationship was obtained between starch lipids and baking quality parameters. These results support the earlier assumption that starch lipids are of no decisive influence upon the baking quality as, in the course of dough preparation, they are not easily accessible to gluten proteins: they are, namely, mainly located in the interior of the starch granules.

Keywords: wheat lipids, wheat starch lipids, baking quality

In the Hungarian agriculture wheat production is of primary importance. The attention of cereal chemists was drawn, in the first place, to the protein of wheats, these were the compounds investigated more thoroughly, as they determine the quality of wheat and of the flour made of it, respectively.

Research performed in the past years has shown that, beside proteins, also other components (lipids, carbohydrates, etc.) and their interactions with proteins, respectively, influence the quality of wheat and flour.

Today, the study and analysis of wheat- or flour lipids forms an important part of cereal chemistry. A number of publications deal with the structure of wheat lipids and the distribution of the main classes of lipids (MECHAM, 1971; MORRISON, 1978; MORRISON et al., 1980; CHUNG & POMERANZ, 1981; POMERANZ & CHUNG, 1983; ZAWISTOWSKA et al., 1984; LÁSZTITY et al., 1987; EMBER-KÁRPÁTI et al., 1987; BÉKÉS et al., 1987; KÁRPÁTI et al., 1990). Two-thirds of wheat endosperm lipids are non-starch-, while one-third are starch lipids. Starch lipids (SL) are structurally bound within the starch granule and can be extracted from flour only after destroying the structure of starch. The other

group of endosperm lipids is formed by non-starch lipids (NSL), which are located outside the starch granule, participate actively in the structure of the gluten skeleton and influence its rheological properties.

In order to gain a thorough knowledge of the lipid composition of starch, both on the macro- and on the molecular level, it is necessary to know also the quantity and composition of starch lipids. Until now, the non-starch lipids of Hungarian wheats were poorly investigated.

In the frame of this publication we give an account on the investigation into starch lipids of Hungarian wheat varieties. This will be the first Hungarian review on the topic.

The lipids to be found in wheat can be attached in different ways to starch granules. On this basis three lipid fractions can be distinguished: true starch lipids, surface lipids of starch and non-starch lipids.

True starch lipids are located within the native starch granules, these are always monoacyl lipids (one fatty acid per molecule). In purified starch the amount of diacyl and triacyl lipids is higher than in crude starch preparations. This indicates that the latter have got into the preparation as impurities (MORRISON, 1988).

Lipids can get also from the surrounding endosperm protein matrix into the hydrated native starch granule, and these are called starch surface lipids. It is not known to what an extent they penetrate the starch granule and how they are bound. It is, however, probable that they can be found in the surface region of the granule, in a form complexed with amylose (MORRISON, 1988).

Starch surface lipids are monoacyl lipids, too, and from the aspect of practice they can be considered as starch lipids as well (MORRISON, 1981). It is assumed that part of the surface lipids originates especially from the aminoplast membrane, which forms part of the protein matrix in nature endosperm (GALLIARD & BOWLER, 1987). At least 90% of starch lipids are lysophospholipids (MEREDITH et al., 1978). Fatty acid composition of wheat starch lipids — according to MORRISON (1988) — is characterized by high content of linoleic (18 : 2) and palmitic acid (16 : 0), while other fatty acids occur only in traces.

Wheat starch might be useful in nutrition as source of essential fatty acids (mainly 18 : 2), as these have been, in general, omitted from foods (MORRISON et al., 1984).

Recently some about fractions of starch lipids were published by MORRISON (1988).

The non-polar lipids occurring in the largest amounts are free fatty acids and the main glycolipids are digalactosyl-monoglycerides, while phospholipids consist mainly of lysophosphatidyl choline.

For the extraction of starch lipids MORRISON (1981, 1988) used various solvents and studied which solvent was the most suited for separating these lipids.

Efficient solvents of starch lipids are 80% diethylene dioxide, 85% methanol at reflux temperature, a mixture of butanol–water (65 : 35, v/v) at 70 °C and water saturated n-butanol at 90–100 °C.

The joint action of water and heat is necessary for swelling of the starch granule, thus making permeation of alcohol and extraction of lipids possible. If the solvent mixture does not contain enough water, swelling of starch will not be sufficient and lipid yield will be low. Although one of the above solvents contains water in the optimum amount, however, when using appropriate parameters, yields above 90% can be achieved.

MORRISON and CONVENTRY (1985) carried out starch lipid extractions with all the alcohols miscible with water. The mixture of n-propanol–water (3 : 1, v/v) proved to be the most efficient solvent, when used for extraction at 90–100 °C, taking 20 cm^3 of solvent for 1 g of starch. Prior to extracting the starch lipids, the free and bound lipids of the samples have to be removed by n-hexane and water-saturated n-butanol extraction (at room temperature), respectively.

1. Materials and methods

1.1. The samples analysed

The samples to be analysed were obtained by courtesy of the Agricultural Institute of Qualification, Budapest, Hungary. The samples originate from the same location: Székkutas, Hungary. The samples analysed are shown in Table 1.

Owing to the small amounts of the samples at our disposal, analyses were carried out with whole meals of wheat samples. (Particle size 0.8–2.0 mm.) Whole meals were obtained by grinding the whole wheat kernels (13.2% moisture content) for one minute in a coffee mill.

1.2. Determination of lipid content and lipid composition

Free lipids were extracted from the whole meal of wheat samples with n-hexane. Ten g samples were shaken in a shaker for 2 h with 50 cm^3 n-hexane. Thereafter, the partially defatted meals were removed from the solvent by filtration (through a filter paper Faltenfilter 100). The solvent was removed from the filtrate by ventilation at room temperature, then the amount of free lipids was determined gravimetrically. All the partially defatted meal samples were shaken again in a shaker for 2 h, using 50 cm^3 water saturated n-butanol at room temperature. After filtration and removal of the solvent, the amount of bound lipids was determined in a similar way as the free lipids.

From the methods published in the literature for starch lipid extraction two were tried by us: extraction with water-saturated butanol at 100 °C and

Table 1

Investigated wheat cultivars

Row	Wheat cultivars
1	Adriana
2	Bucsányi 20
3	GK Bence
4	GK István
5	GK Kincső
6	GK Öthalom
7	GK Zombor
8	Yubileynaya 50
9	M 3
10	Mv 9
11	Mv 12
12	Mv 14
13	Mv 15
14	Mv 16
15	Vitka

extraction with a n-propanol – water (3 : 1, v/v) mixture at 90–100 °C (MORRISON, 1981, 1989). According to both the data from the literature and our own experiments the second method proved more suited for the extraction of these lipids, thus this was used further on.

After removal of the free and bound lipids, starch lipids were extracted from all the meal samples on a water bath, using a mixture of n-propanol – water (3 : 1, v/v). After filtration and removal of the solvent, the amount of starch lipids was determined in a way similar to that used with free and bound lipids.

1.3. Chromatographic separation methods

Fractionation of starch lipids was carried out on a micro-column filled with 100–120 mesh Florisil (Magnesium-silicate) pretreated with hydrochloric acid. Hydrochloric acid pretreatment of the column filling was carried out according to the method described by KATES (1972). The size of the column was 0.4 cm × 13 cm, glass wool was put on its bottom and it was filled to a height of 10 cm with Florisil filling dried overnight. A solution containing, on the average, 40–50 mg lipids was applied to the column, whereby the lipids were dissolved in 0.5 cm^3 n-propanol – water (3 : 1).

Three lipid fractions were separated from the starch lipids, adapting the method of BÉKÉS and co-workers (1983). First the non-polar lipids were eluted from the column with 15 cm^3 chloroform, then the glycolipids with 15 cm^3 ace-

tone and finally, the phospholipids with 15 cm^3 methanol. From the fractions thus obtained, the solvents were removed at room temperature by ventilation, then the amounts of the individual fractions were determined by weighing on an analytical balance. Two parallel samples were used for the determinations and the results were given in mg related to 100 g meal.

The lipid fractions obtained from the starch lipids by column chromatography were further separated by the thin-layer-chromatographic method applied by MORRISON and co-workers (1980). "Polygram oil G" (Macherey–Nagel and Co.) thin-layer-chromatographic plates of 10×10 cm were used for the separation.

The thin-layer-chromatographic plates were developed, for photographic purposes, in a desiccator filled with iodine vapours. Quantitative evaluation was carried out using data from the literature (MORRISON et al., 1980) and was based on R values (retention factors). For quantitative evaluation a sulfuric acid reagent was used (45% sulfuric acid), and the plates were kept in a 120 °C oven till the appearance of the spots.

1.4. Investigation of baking properties

Hagberg number, Valorigraph value, water absorption capacity and shape ratio of samples were determined using the Hungarian standard methods (HUNGARIAN STANDARD, 1973, 1978). Baking tests were performed based on the method of the HUNGARIAN STANDARD (1978), as modified by MOOR (1975). Characterization of samples for baking properties were done in the laboratories of the Hungarian Research Institute of Milling.

2. Results and evaluation

2.1. Lipid content and lipid fraction distribution

The averages of the results of three parallel measurements of free (FL), bound (BL), starch (SL), starch non-polar (SNPL), starch glyco-(SGL) and starch phospholipids (SPHL) are summarized in Table 2.

The amounts of free lipids varied between 683.5 and 1001.0 mg per 100 g meal in the wheat samples investigated. The mean value was 856.8 mg per 100 g meals and the standard deviation of the values was 88.96. The percentage relative standard deviation (CV) was 1.2%. The amount of bound lipids varied from 809.3 to 1417.0 mg per 100 g meal. The mean value was 1024.4 mg per 100 g meal, standard deviation was 148.06 and CV = 1.2%. The amount of starch lipids varied between 761.7 and 1196.4 mg per 100 mg meal. The mean value was 967.6 mg per 100 g meal, standard deviation was 135.35 and

CV = 1.3%. For non-polar starch lipids the values varied between 42.3 and 98.9 mg per 100 g meal. The mean value was 65.9 mg per 100 g meal, standard deviation was 18.99 and CV = 3.8%. The amount of starch glycolipids varied between 14.3 and 43.2 mg per 100 g meal, the mean value was 27.1 mg per 100 g meal, standard deviation was 8.53 and CV = 6.2%. For starch phospholipids the values were between 690.7 and 1096.7 mg per 100 g meal. The mean value was 874.6 mg per 100 g meal, standard deviation was 125.20 and CV = 1.0%.

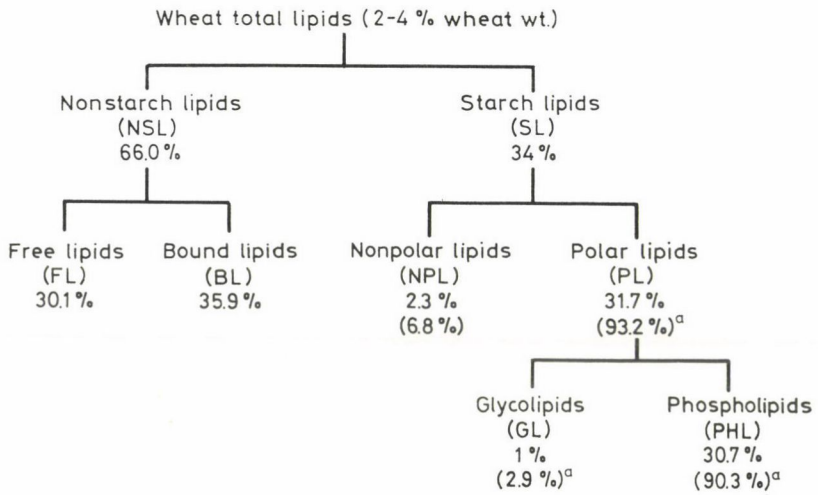

Fig. 1. Lipid composition of wheat (whole meal) lipids. [a] The data as related to starch lipid content

Figure 1 shows the mean lipid composition obtained from the data of the samples as related to total lipid content and the average starch lipid composition.

The lipid data show a very good agreement with the data published in the literature both for non-starch (NSL) and for starch lipids (SL).

From the standard deviations of the parallel measurements it can be seen that extraction methods and column chromatographic separation gave well reproducible results.

2.2. Components of the lipid fractions

The results obtained by thin-layer-chromatographic separation of the starch lipid fractions are shown in Table 3.

In the cease of non-polar lipids, free fatty acids occurred in the largest amounts in every case. The individual lipid classes were, in decreasing order of quantities, as follows:

$$FFA > MG = TG > DG = SE$$

Table 2

Lipid contents of whole meals
(mg lipid per 100 g dry weight)

Wheat cultivars	FL	BL	SL	SNPL	SGL	SPHL
Adriana	954.3	972.7	814.3	53.9	17.1	744.6
Bucsányi 20	688.1	1059.3	1164.7	56.0	15.9	1092.8
GK Bence	864.3	870.8	1071.2	84.2	42.0	945.0
GK István	815.6	1115.3	970.5	43.5	19.2	907.8
GK Kincső	853.5	974.4	871.8	54.0	35.1	782.7
GK Öthalom	979.6	1118.2	772.0	52.9	16.7	702.4
GK Zombor	817.9	1230.0	887.5	50.2	32.6	804.7
Yubileynaya 50	864.6	975.3	1185.3	98.1	26.3	1060.9
M 3	911.7	1407.9	905.6	49.1	25.1	831.4
Mv 9	760.4	931.9	1048.9	63.0	40.8	945.1
Mv 12	900.2	1064.5	807.9	91.0	22.0	694.8
Mv 14	882.9	896.8	1168.1	90.0	28.9	1049.2
Mv 15	856.0	956.7	1023.8	92.5	35.5	895.8
Mv 16	705.9	812.8	957.9	57.7	21.7	878.5
Vitka	997.7	978.9	864.2	52.6	27.2	784.4

Data are averages of two parallel measurements

In the case of glycolipids, digalactosyl-monoglycerides occurred in the largest amounts, the further order of quantities was:

$$DGMG > MGMG = DGDG > MGDG$$

The phospholipids are mainly lysophospholipids, and lysophosphatidylcholine was found in the largest amount. The quantitative order of the individual lipid classes was:

$$LPC > LPE > LPG > LPI + PA$$

The results obtained by us are in good agreement with the data found in the literature (MORRISON, 1978, 1988).

The correlation coefficients of the relationships between the lipid data were calculated by computer. The correlation matrix is shown in Table 4. From this it is visible that good correlations can be found between the following parameters:

free lipids – starch lipids	$r = -0.533$
free lipids – starch phospholipids	$r = -0.577$
starch lipids – starch phospholipids	$r = 0.987$

The relationships between the above parameters have been determined also in the form of specific equations, using the method of linear regression. The parameters of the equations are given in Table 5.

Table 3

Starch lipids compositions and contents in wheats by thin-layer chromatography
(mg per 100 g)

Lipid class	Lipid contents
NONPOLAR LIPIDS (NPL)	
Monoglycerides (MG)	9–20
Free fatty acids (FFA)	33–73
Diglycerides (DG)	0–11
Triglycerides (TG)	5–18
Steryl esters (SE)	4–10
GLYCOLIPIDS (GL)	
Digalactosyl monoglycerides (DGMG)	11–49
Digalactosyl di-glycerides (DGDG)	6–13
Monogalactosyl monoglycerides (MGMG)	4–16
Monogalactosyl di-glycerides (MGDG)	0–10
PHOSPHOLIPIDS (PHL)	
Lysophosphatidyl inositols (LPI)	
Phosphorous acids (PA)	9–38
Lysophosphatidyl choline (LPC)	567–879
Lysophosphatidyl ethanolamines (LPE)	76–117
Lysophosphatidyl glycerols (LPG)	21–46

Table 4

Simple correlation coefficients between lipid data and baking quality parameters

	FL	BL	SL	SNPL	SGL	SPHL
Free total lipid	1.000					
Bound total lipid	0.218	1.000				
Starch total lipid	−0.533*	−0.352	1.000			
Starch nonpolar lipid	0.049	−0.437	0.498	1.000		
Starch glycolipid	−0.088	−0.276	0.268	0.343	1.000	
Starch phospholipid	−0.577*	−0.296	0.987***	0.364	0.170	1.000
Wet gluten content	0.026	−0.328	0.205	0.202	0.099	0.185
Spreading of wet gluten	0.002	−0.255	0.051	0.132	0.010	0.034
Water absorption	−0.453	−0.002	0.348	0.104	0.136	0.352
Valorigraph value	−0.204	0.159	0.341	0.339	0.050	0.314
Hagberg falling number	0.288	0.098	−0.016	0.153	0.248	−0.057
Loaf volume	−0.152	0.344	−0.013	0.048	−0.063	−0.017
Form ratio of loaf	0.540*	−0.351	0.004	0.321	−0.036	−0.041

* Significant at $P \leq 0.05\%$ probability level
*** Very highly significant at $P \leq 0.001$ probability level
$n = 15$

Table 5

Parameters of linear regression ($y = ax + b$) of lipid data

Y	X	A	B
Free total lipid	Starch total lipid	—0.350	1195.37
Free total lipid	Starch phospholipid	—0.409	1214.88
Starch total lipid	Starch phospholipid	1.066	34.94

Table 6

Simple correlation coefficients between normalized lipid data and baking quality parameters

	FL	BL	SL	SNPL	SGL	SPHL
Free total lipid	1.000					
Bound total lipid	0.302	1.000				
Starch total lipid	—0.614**	—0.354	1.000			
Starch nonpolar lipid	0.018	—0.415	0.448	1.000		
Starch glycolipid	—0.016	—0.151	0.334	0.347	1.000	
Starch phospholipid	—0.668**	—0.307	0.985***	0.302	0.230	1.000
Wet gluten content	—0.123	—0.411	0.065	0.130	0.047	0.047
Spreading of wet gluten	—0.036	—0.260	—0.004	0.103	—0.012	—0.020
Water absorption	—0.569	—0.098	0.248	0.049	0.094	0.253
Valorigraph value	—0.334	0.044	0.253	0.305	0.008	0.225
Hagberg falling number	0.213	0.047	0.082	0.133	0.233	—0.128
Loaf volume	—0.137	0.319	0.005	0.070	—0.049	—0.002
Form ratio of loaf	0.417	—0.399	—0.103	0.279	—0.076	—0.150

* Significant at $P \leq 0.05\%$ probability level
** Highly significant at $P \leq 0.01\%$ probability level
*** Very highly significant at $P \leq 0.001\%$ probability level
$n = 15$

According to data from the literature, environmental effects can be eliminated to a certain extent, if data are related to standard protein content (CHUNG et al., 1982). The investigation into the relationships was, therefore, carried out also for lipid data related to standard protein content. The correlation matrix of these data is contained in Table 6.

The best correlation coefficients were found between the following data:

free lipids – starch lipids	$r = -0.614$
free lipids – starch phospholipids	$r = -0.668$
starch lipids – starch phospholipids	$r = 0.985$

The parameters of the equations determined by linear regression calculation are to be found in Table 7.

Table 7

Parameters of linear regression ($y = ax + b$) of normalized lipid data

Y	X	A	B
Free total lipid	Starch total lipid	—0.461	92.46
Free total lipid	Starch phospholipid	—0.542	94.42
Starch total lipid	Starch phospholipid	1.065	2.58

Table 8

Data of baking quality parameters

	Wet gluten content (%)	Spreading of wet gluten (mm h^{-1})	Water absorption (Valorigraph) (%)	Valoriograph value	Hagberg falling number (s)	Loaf volume (cm^3 per 360 g flour)	Form ratio of loaf
Adriana	36.0	12.5	55.4	30.5	356	840	2.0
Bucsányi 20	28.5	3.5	58.2	62.6	278	970	1.8
GK Bence	31.3	7.0	58.8	61.7	335	950	1.9
GK István	34.0	6.0	62.5	71.2	426	1010	1.9
GK Kincső	32.1	6.5	56.6	40.7	376	870	1.9
GK Öthalom	31.9	5.5	60.2	66.7	393	1040	2
GK Zombor	34.3	9.5	62.5	55.1	386	920	1.8
Yubileynaya 50	34.0	3.5	60.2	75.6	442	1010	1.9
M 3	31.6	4.0	62.7	74.6	336	1040	1.8
Mv 9	33.5	2.5	69.8	74.6	440	1090	1.9
Mv 12	30.2	3.5	58.9	74.6	374	1070	1.9
Mv 14	39.7	14.0	65.6	63.5	370	860	2.1
Mv 15	34.5	9.5	61.4	68.8	361	930	1.9
Mv 16	35.5	9.0	66.1	67.5	282	950	1.8
Vitka	31.0	1.0	56.3	62.0	359	910	1.9

The closest correlation was found in both cases between starch lipids and starch phospholipids (r = 0.987 and 0.985). According to the results of column chromatographic separation, 90% of starch lipids consisted of phospholipids. This explains the close correlation.

Table 8 shows data of baking quality parameters (wet gluten content, spreading of wet gluten, Hagberg falling number, Valorigraph value, Valorigraph water absorption, loaf volume, form ratio of loaf).

The correlation between baking quality parameters and the different lipid fractions were also studied. The results of calculations are included in Tables 4 and 6.

For the wheat samples investigated by us no relationship could be detected between starch lipids and baking quality parameters. These results support the earlier assumption that starch lipids are of no decisive influence

upon baking quality as, in the course of dough preparation, they are not easily accessible to gluten proteins, being located mainly in the interior of the starch granules.

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A STUDY ON THE COOKING AND EATING QUALITY CHARACTERS OF SOME EGYPTIAN RICE VARIETIES

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Research was conducted to evaluate some cooking and sensoric quality characters of 10 Egyptian rice varieties and new strains during 1989 season. These traits were grain length, grain width, grain shape (L/W ratio), gelatinization temperature (GT), gel consistency (GC), amylose content and protein content beside, sensory test by using 10 judges. These tests were rice:water ratio, cooking time, kernel expansion, breakage percentage, whiteness, hardness, stickiness, odour and taste.

The results showed that the short grain varieties had lower amylose content than the long ones, while insignificant, differences were obtained in the other traits. The short grain varieties required less amount of water and less cooking time. Significant correlation coefficients between amylose content and rice:water ratio, cooking time, hardness and stickiness were also obtained. Different values of correlation coefficients between the studied traits were estimated. In general the study showed that the short grain varieties with low amylose content may have more attractive taste than the long grain with high amlyose content for the Egyptian consumers.

Keywords: rice, eating quality of rice, cooking quality of rice, Egyptian rice

Rice is one of the major field crops in Egypt. It is a crucial commodity of the nation. It is an essential food crop preferred by a large group of the population in comparison to any other carbohydrate rich foods.

Cooking and eating quality of rice has never been serious problem in Egypt since nearly more than 95% of the rice area is planted by japonica rice varieties because of their moistness, tenderness, gloss and taste. Recently, however, emphasis of development of long grain indica rice has brought into focus the problem of cooking and eating quality in breeding program. Newly released indica rice (IR 28) and indica japonica hybrid (Giza 175) are mostly of high amylose content which cook dry and hard, and are therefore not acceptable to the local consumers. As a result, the extension of these cultivars has been adversely affected, even though they generally have higher yield potential and are resistant to blast, the major disease in Egypt.

Inadequate basic information on cooking and eating quality of the commercial rice varieties grown in Egypt are available. Keeping in view the need of such information, the present investigation was undertaken to study the

cooking and eating quality of some Egyptian rice varieties and new lines depending on the dietary habit and the manner in which rice is cooked and consumed in Egypt.

1. Materials and methods

1.1. Materials

This experiment was conducted at Rice Research and Training Center (RRTC) Sakha Kafr, El-Sheikh, Egypt, during 1989 season. The study was aimed to investigate the cooking and eating quality characters of some Egyptian rice varieties and strains. These characters were gelatinization temperature (GT), gel consistency (GC) and amylose content as chemical characters. Besides some other cooking and eating quality traits namely, rice:water ratio, cooking time, kernel expansion, breakage percentage after cooking, whiteness, cooked kernel hardness, stickiness, odour and taste, were tasted according to the dietary habit and the manner in which rice is cooked and consumed in Egypt. In addition protein content was also tested.

Ten Egyptian rice varieties and strains were used in this study. These varieties were Giza 171, Giza 172, Giza 175, Giza 159, Reiho and Gz 2175–5–6 as short grain Giza 181, IR 28 and IR 19743–46, as long grain, and GZ 1368 S–5–2 as medium grain.

1.2 Determination of cooking and eating quality characters

Samples of the studied varieties were dehulled in Satake testing husker and polished in Dayton milling machine at the Grain Quality Lab., RRTC Sakha, Egypt. The specific physico-chemical tests were used to determine the cooking and eating quality according to LITTLE and co-workers (1958), JULIANO (1971), CAGAMPANG and co-workers (1973), AZEEZ and SHAFI (1966) and IRRI (1970). On the other hand, 100 g of milled rice samples were cooked and were served to a panel of 10 judges for evaluation. The judges were instructed to sip water before and after tasting each sample. The samples were evaluated for rice : water ratio, cooking time, kernel expansion, breakage percentage after cooking, whiteness, cooked kernel hardness, stickiness, odour and taste according to PERYAM and SHAPIRO (1955). The samples were evaluated using ten points scale for each character with maximum scores of 70 and a limit of acceptability of 50 scores. Protein content was estimated following A.O.A.C. method (1975).

Correlation coefficients between some physico-chemical properties of rice grain and all the other studied traits according to TOMAR (1981) were also determined.

2. Results and discussion

2.1. The physico-chemical properties of rice grain

Table 1 shows the means of physico-chemical properties of the rice grains for the before mentioned Egyptian varieties. The protein content of these varieties is also included. The short grain varieties ranged between 4.95 and 5.64 mm in length and from 2.36 to 2.92 mm in width, while the long grain ones ranged between 6.02 and 6.63 mm in length and from 2.13 to 2.16 in width. These measurements in turn affected the grain shape (length/width ratio) which varied from 1.73 to 2.30 mm in case of the short grain varieties and from 2.81 to 3.11 mm for the long grain varieties. The lowest value of grain shape (1.73 mm) was determined for Reiho variety, while the highest value (3.11 mm) was measured for Giza 181 variety.

Table 1

Means of physico-chemical properties of grain for ten Egyptian rice varieties and strains

Variety	Grain length (mm)	Grain width (mm)	Grain shape (mm)	Gelatinization temperature	Gel consistency	Amylose content (%)	Protein content (%)
Giza 171	5.64	2.42	2.19	5	91	19.45	6.3
Giza 172	5.21	2.82	1.84	6	95	19.7	7.2
Giza 159	4.95	2.81	1.76	6	100	20.05	7.1
Giza 175	4.97	2.41	2.11	3	36	25.51	8.3
Reiho	5.06	2.92	1.73	7	100	18.11	6.6
Gs 2175-5-6	5.14	2.68	1.92	7	100	18.91	6.2
Giza 181	6.02	2.15	2.18	5	82	20.71	7.6
IR 28	6.63	2.13	3.11	1	37	27.11	8.2
IR 19743-46	6.46	2.16	2.99	1	38	26.31	10.1
GZ 1368-S-5-2	5.43	2.36	3.21	4	32	26.11	7.6

Cooking and eating quality of rice is generally measured by gelatinization temperature (GT), gel consistency and amylose content. In the present investigation, gelatinization temperature ranged between 1 (for IR 28 and IR 19743–46) to 7 (for Reiho and GZ 2175–5–6). These results indicate that Reiho and GZ 2175–5–6 rice varieties are less resistant and take short time for cooking. On the other hand the lowest gel consistency (GC) scores were recorded for the varieties GZ 1368–S–5–2, Giza 175, IR 28 and IR 19743–46. These scores were 32, 36, 37 and 38 mm for these rice varieties, respectively. While, the highest GC scores (100 mm) were recorded for Giza 159, Reiho and GZ 2175–5–6 rice varieties. These findings indicated that the indica type varieties showed lower values of GC than the japonicas. These results were in agreement with those reported by JULIANO et al. (1964).

The amylose content varied from 27.1% (for IR 28) to 18.1% (for Reiho). It is clear, from the data presented in Table 1 that all the short grain varieties had low amylose content except the variety Giza 175. While, the long grain varieties had a high amylose content. This result indicates that the japonica rice varieties under this study were more sticky than the indicas when cooked. Similar results were reported by KUO and HSIEH (1981).

In regard to protein content, the highest value was determined for IR 19743–46 and the lowest one was found for GZ 2175–5–6 (Table 1). These percentages were 10.1 and 6.2 for the two rice varieties, respectively. The indica type varieties showed higher protein percentage (7.6–10.1%) than the japonicas (6.2–8.3% is in agreement with the findings obtained by KAMBAYASHI and coworkers (1989).

2.2. The panel test characters

According to the total score recorded by the ten judges, panel test characters are presented in Table 2. Data presented revealed that the rice–water ratio was 1 : 1 for all the short grain varieties except the variety Giza 175, 1 : 1.5 for the long grain varieties IR 28 and IR 19743–46 and 1 : 2 for Giza 181, only. These results were in agreement with those reported by EL-KADY and KASSEM (1989).

The cooking time varied with the rice–water ratio. With 1 : 1 ratio the cooking time ranged between 19 min (Reiho) and 33 min (IR 28) and from 21 min to 32 min for Gz 2175–5–6 and IR 28, respectively, with 1 : 1.5 ratio. While, with 1 : 2 ratio IR 28 also had the highest (25 min) and Giza 171 the lowest (19 min) cooking time.

The expansion of the grains after cooking was the highest for Giza 171 and GZ 2175–5–6, while the lowest was recorded for the two rice varieties Giza 175, IR 28 and Giza 181. Moreover, the breakage percentage as shown in Table 2 was the highest for all the tasted varieties when the rice–water ratio was increased. IR 28 variety had the highest breakage percentage followed by IR 19743–46, while the lowest was recorded for Giza 172 and GZ 2175–5–6 rice varieties.

The mean score for whiteness was the highest for cooked Giza 181 and the lowest for Giza 175. The differences in whiteness between the other varieties were not significant according to the acceptable score. The acceptable score for hardness was the lowest for Giza 175 (4.5) and highest for Giza 172 (9) (Table 2).

Data in Table 2 revealed that stickiness increased with increasing rice–water ratio for the short grain varieties which indicated that these varieties do not need an increased amount of water during cooking, according to the opinion of the judges. Moreover, varieties Reiho, Giza 171, Giza 172 and Giza

Table 2

Means of panel test characters of some Egyptian rice cultivars and strains

Varieties	Rice-Water	Cooking time (min)	Ex-pansion	Break-age	White-ness	Hard-ness	Sticki-ness	Odor	Taste	Total score
	1 : 1	20.00	9.00	8.00	9.00	7.00	8.00	9.00	9.00	59.00
Giza 171	1 : 1.5	22.00	8.00	7.00	9.00	6.00	8.00	9.00	8.00	55.00
	1 : 2	19.00	5.00	5.00	9.00	5.00	5.00	8.00	7.00	44.00
	1 : 1	23.00	8.00	9.00	9.00	8.00	8.00	9.00	9.00	60.00
Giza 172	1 : 1.5	22.00	7.00	8.00	9.00	9.00	7.00	9.00	8.00	57.00
	1 : 2	22.00	6.00	6.00	9.00	5.00	6.00	8.00	7.00	47.00
	1 : 1	20.00	7.00	8.00	7.00	7.00	7.00	8.00	7.00	51.00
Giza 159	1 : 1.5	24.00	6.00	6.00	7.00	6.00	6.00	8.00	7.00	46.00
	1 : 2	23.00	5.00	7.00	7.00	6.00	6.00	8.00	5.00	44.00
	1 : 1	22.00	4.50	8.00	6.50	5.00	5.00	6.50	4.50	40.00
Giza 175	1 : 1.5	22.00	4.50	8.00	6.50	5.00	5.00	6.50	5.50	41.50
	1 : 2	21.00	4.50	5.50	7.00	4.50	4.50	7.00	4.50	37.50
	1 : 1	19.00	8.00	8.00	9.00	8.00	8.00	9.00	9.00	59.00
Reiho	1 : 1.5	23.00	7.00	8.00	9.00	7.00	6.00	9.00	7.00	53.00
	1 : 2	22.00	7.00	6.00	9.00	6.00	5.00	9.00	6.00	48.00
	1 : 1	20.00	9.00	9.00	9.00	8.00	8.00	8.00	9.00	60.00
GZ 2175	1 : 1.5	21.00	8.00	7.00	9.00	7.00	7.00	7.00	7.00	52.00
	1 : 2	21.00	6.00	5.00	9.00	6.00	6.00	7.00	6.00	45.00
	1 : 1	23.00	5.00	7.00	10.00	7.00	6.00	9.00	6.00	50.00
Giza 181	1 : 1.5	25.00	6.00	7.00	10.00	6.00	7.00	9.00	8.50	53.50
	1 : 2	20.00	7.00	6.00	10.00	6.00	8.00	10.00	9.00	57.00
	1 : 1	33.00	5.00	7.00	8.00	7.00	4.00	6.00	5.00	42.00
IR 28	1 : 1.5	32.00	7.00	6.00	8.00	6.00	6.00	7.00	7.00	47.00
	1 : 2	25.00	6.00	5.00	8.00	6.00	5.00	7.00	6.00	43.00
	1 : 1	23.00	7.00	7.00	8.00	7.00	6.00	5.00	4.00	44.00
IR 19743	1 : 1.5	22.00	8.00	6.00	8.00	6.00	8.00	6.00	6.00	48.00
	1 : 2	23.00	8.00	6.00	8.00	6.00	7.00	6.00	5.00	46.00
	1 : 1	24.00	7.50	8.00	7.00	8.50	7.50	8.00	8.00	53.00
GZ 1368	1 : 1.5	24.00	6.50	5.50	7.00	7.50	6.50	8.00	7.00	48.00
	1 : 2	22.00	6.00	6.00	7.00	6.00	5.00	8.00	6.00	44.00

181 were the most acceptable varieties due to their odour and taste, while the lowest acceptable varieties were Giza 175 and IR 19743–46.

The overall acceptability using a 70 points scale ranged between 60 and 37.5. The most acceptable varieties were Giza 172 followed by GZ 2175–5–6, while the lowest were Giza 175, IR 28 and IR 19743–46.

2.3. The correlation coefficients between the physico-chemical properties and the cooking and eating quality characters

Table 3 showed that rice–water ratio was positively correlated with gelatinization temperature (GT) and amylose content only. These results revealed that rice with high amylose absorbed more water during cooking compared to rice with low amylose. THENAMMI and co-workers (1975), SOOD (1978), TOMAR and NANDA (1982) and MADAN and BHAT (1984), reported similar findings.

Negative and significant correlation coefficients were estimated between cooking time and grain length, gelatinization temperature (GT), and gel consistency (GC). While highly significant positive correlation was found between cooking time and amylose content. JULIANO and GONZALES (1987) mentioned that, cooking time, the period required for the grain core to be gelatinized (absence of apaque center) in boiling water, is affected directly by GT.

The data represented in Table 3 indicated that kernel expansion was positively correlated with GT, GC and amylose content. MADAN and BHAT (1984) reported that the elongation of rice during cooking might be dependent on variety and duration of storage of rice. Moreover a significant positive correlation coefficient was obtained between amylose content and elongation ratio of rice. Thus, rice with high amylose content may increase rather in length during cooking than rice with low amylose. Some results were reported by JULIANO and GONZALES (1987).

Breakage percentage after cooking was positively correlated with grain length and cooked grain whiteness was correlated with amlyose content (Table 3). Moreover, hardness of the grains after cooking was positively correlated with grain length, GT, GC, amylose content and protein content. Similar results were reported by RAGHAVAIAH and KAUL (1970) and MADAN and BHAT (1984). Cooked rice hardness was measured by JULIANO and GONZALES (1987) using a 10 cm^2 Ottawa Texture measuring system. They reported that the hardness value is affected not only by amylose content, but also by gel consistency and protein content.

Stickiness was positively correlated with GC and negatively with amylose content. Same findings were suggested by JULIANO and GONZALES (1987). Positive and significant correlation coefficients were estimated between taste and grain shape and GC. While the correlation was negative with grain length and amylose content. Thus, rice of short grain and low amylose content may have more acceptable taste than rice with high amylose.

Thus it can be concluded from the present study that Giza 172 rice variety and the newly developed strain GZ 2175–5–6 were the best from the point of view of cooking quality and acceptability. Moreover, amylose content is the most important index of eating quality.

Table 3

Correlation coefficients between the physiocochemical properties and some cooking and eating quality characters of rice grain

Characters	Grain length	Grain width	Grain shape	Gelatinization temperature	Gel consistency	Amylose content	Protein content
Rice–water ratio	0.093	0.001	0.023	0.311*	0.033	0.631**	0.127
Cooking time (min)	—0.217*	0.176	—0.224	—0.543**	—0.368*	0.445**	—0.0278
Kernel expansion	0.107	0.193	0.159	0.298*	0.257*	0.290*	0.165
Breakage (e)	0.309*	0.05	0.077	—0.123	0.243	0.031	—0.009
Whiteness	0.007	—0.203	—0.168	0.065	0.088	—0.277*	0.113
Hardness	0.264*	0.088	0.236	0.644**	0.491**	0.368**	0.291*
Stickiness	—0.193*	0.12	—0.007	—0.048	0.432**	—0.534**	0.031
Odor	0.131	0.215	0.113	0.123	0.003	0.011	0.052
Taste	—0.188*	—0.136	0.287*	0.15	0.434*	—0.263*	0.219

* Significant at P = 0.005 probability level
** Significant at P = 0.01 probability level

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FUNCTIONAL PROPERTIES OF SOME SUCCINYLATED PROTEIN PREPARATIONS

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Three different protein preparations: wheat gluten, zein and egg albumin were modified using succinic anhydride and protein at a ratio of 3 : 1 (w/w). Comparing the three proteins investigated it was stated that according to the extent of modification (succinylation) the following ascending order was observed: gluten, zein, egg albumin. Improvement in the protein solubility, emulsifying capacity and foam properties was more significant in the case of succinylated gluten than the succinylated other two types of proteins especially egg albumin.

Keywords: protein, gluten, zein, egg albumin, chemical modification of proteins, succinylation of proteins

The use of succinic anhydride, as a modifying agent of proteins, was first studied by HABEEB and co-workers (1958). Since then, the technique has been developed and discussed by several authors (HASS, 1964; KINSELLA & SHETTY, 1979; VANANUVAT & KINSELLA, 1976; MA et al., 1986). Recently a review of possible chemical modifications was given by HALÁSZ and LÁSZTITY (1991). Succinylation of amino acid residues, e.g. and ε-amino groups, has three major effects on the physical characteristics of proteins, it increases net negative charge, change conformation and the propensity of proteins to dissociate into subunits (GOUNARIS & PERLMANN, 1967; GIRDHAR & HANSEN, 1974).

The introduction of succinate anions alters the conformation of proteins and the penetration of water molecules into the protein becomes easier because of the loosened state of the polypeptides (KINSELLA & SHETTY, 1979; HALÁSZ & LÁSZTITY, 1986).

Many studies have been conducted on the succinylation of different proteins, using some different reaction conditions, and obtaining different rates of succinylation. The rate of succinylating fish myofibrillar protein was 77% when the reaction conditions were: 0 °C, pH 7.5–8.5 and 1 : 5 succinic anhydride–protein ratio (GRONINGER, 1973). Wheat gluten was succinylated to 60% of its free amino group when the ratio of succinic anhydride gluten was 1 : 2 (BARBER & WARTHESEN, 1982). A higher rate of gluten succinylation (95% was produced when dioxane was used as a solvent of succinic anhydride reagent (GRANT, 1973). Egg albumin reached complete succinylation 100% at a ratio of 1 : 10 anhydride : protein (MA & HOLME, 1982) while β-casein reached 86%

succinylation at 1 : 2 anhydride : protein (HAAGLAND, 1968). DOHEEM and co-workers (1988) modified wheat gluten by succinic anhydride at different ratios 1 : 2, 1 : 1, 3 : 1 and 4 : 1 reagent (gluten w/w) and found that the highest rate of modification (40.8% succinylation) was obtained when the reagent was added at a ratio of 3 : 1 as dissolved in dioxane.

These results show that the rate of succinylation is highly depending on the quality of proteins and also on the reaction conditions.

To compare the capacity of different proteins, resp., protein preparations to the succinylated, succinylation reaction conditions and the methodology for determining the succinylation rate must be the same in each case. The present study is comparing the succinylation rate of three different proteins, wheat gluten, zein and egg albumin under the same conditions. In addition functional properties of these modified proteins were investigated.

1. Materials and methods

Wheat gluten (75% protein), egg albumin (98% protein) and pure corn zein (96% protein) were obtained from Sigma Co. (Germany). Commercial corn zein (65% protein) was obtained from Starch and Glucose Factory, Mostorod, Egypt.

1.1. Preparation of succinylated proteins

Three samples of pure proteins, wheat gluten, zein and egg albumin as well as commercial zein were succinylated under the same conditions; the ratio of succinic anhydride–protein was 3 : 1 and the reagent was added as dissolved in dioxane. All the other reaction conditions were described by BARBER and WARTHESEN (1982). Protein sample was dispersed in water adjusted to pH 8.5 with NaOH (1 *N*). Succinic anhydride was added (3 : 1 ratio) then the pH was readjusted with NaOH (3.5 *N*). When acylation was completed, the solutions were exhaustively dialyzed against distilled water (4 °C for 48 h) to remove impurities and excess reagents. The resulting fractions were concentrated under vacuum and finally dried at 40 °C until complete dryness. Extent of modification was followed by determining the free amino groups before and after succinylation using TNBS (trinitro benzene sulfonate) method of HABEEB (1966).

1.2. Protein solubility

Modified proteins and the unmodified one were dispersed in water (0.1% w/v) and pH was adjusted in the range 3–8 with either HCl conc. or NaOH 3 *N* solutions. The resulting solutions were centrifuged at 3000 r. p. m.

for 15 min. The supernatant was filtered through filter paper Whatman No. 1. Protein content was determined in the filtrate by the method of LOWRY and co-workers (1951) with bovine serum albumin as a standard. Protein solubility was expressed as the percentage of total protein concentration:

$$\text{Protein solubility} = \frac{\text{Amount of soluble protein}}{\text{Amount of total protein}} \times 100$$

1.3. Emulsifying capacity (EC)

Aqueous dispersions of the unmodified and modified proteins were adjusted to pH 9.5 and pH 7.5, respectively and used for the determination of emulsifying capacity according to the procedure of FAYED (1987). The emulsifying capacity was expressed as cm^3 oil per gram protein.

1.4. Foaming properties

Foaming capacity and stability were determined in the aqueous dispersion of unmodified and modified protein being adjusted to pH 9.5 and 7.5, respectively, according to FAYED (1987). Foam overrun was calculated as:

$$\text{Foam overrun} = \frac{\text{Volume after whipping-Volume before whipping}}{\text{Volume before whipping}} \times 100$$

Foam stability was followed by measuring the decrease in foam volume with time.

2. Results and discussion

2.1. Extent of modification

Comparing the protein preparations it was found that egg albumins had the highest rate of succinylation (84%) while gluten preparation showed the lowest one (40.8%). Zein showed a medium rate (67.0%).

These differences between the different proteins in the rate of succinylation under same conditions are connected with several factors. Solubility plays an important role in the reactivity of the protein molecule and its accessibility for the succinylation process as indicated by BERBER & WARTHESEN (1982). So, the high extent of egg albumin modification may be due to their complete solubility at the reaction pH. The fact that most of the hydrophobic amino-acid residues located in the hydrophobic core of globular molecule and polar amino group mainly at the surface region may be also important. The value found is in accordance with that reported by MA and HOLME (1982).

On the other hand poor solubility of gluten might explain partly its poor modification rate as concluded by BARBER and WARTHESEN (1982). In addi-

tion the complex nature of the gluten complex, interactions with other compounds (lipids, carbohydrates, phytic acid) present in gluten are also hindering factors. Zein is comparatively more soluble at the reaction pH = 8 than gluten (Fig. 1). This explains the superiority of zein to gluten in the rate of succinylation. However, commercial zein showed a very poor rate of succinylation. The clear difference between commercial and pure zein may be due to the presence of lipids and carbohydrates interacting with protein molecules.

2.2. Solubility profiles of different succinylated proteins

The data in Fig. 1 show the pH-solubility curves of different succinylated protein preparations as compared to their unmodified forms. It may be noted that all the studied proteins exhibited enhanced solubilities at the basic pH of their pH-solubility curves compared to their unmodified forms. The highest increase was observed in modified gluten.

Also, all succinylated proteins except egg albumin showed reduced solubility values at the acidic pH of their pH-solubility curves when compared with their unmodified forms.

BARBER and WARTHESEN (1982) explained the reduced solubility of the succinylated proteins at the acidic pH on the basis that the elimination of the positive charges (from the free amino groups) and repression of dissociation of carboxyl groups had led to an insufficient number of hydrophilic groups at pH 3 to overcome the aggregating forces resulting from the hydrophobic bonds. The reducing effect of succinylation on protein solubility at the acidic pH is quite in accordance with the results of BARMAN and co-workers (1977) working on soybean protein.

On the other hand increase of solubility at alkaline pH-s is the consequence of increasing the number of negatively charged ionizable groups such as carboxyl groups.

The difference between zein and the low protein commercial preparation is a direct result of the difference in the extent of succinylation.

Egg albumin showed the least response to the succinylation treatment. This solubility behaviour can be explained with different molecular structure and composition of this protein containing phosphate groups, carbohydrates, higher amount of lysine and free (non amidated) carboxyl groups. It can be mentioned that MA and HOLME (1982) found that the surface hydrophobicity of egg albumin remained constant upon succinylation, i.e., no extensive unfolding or rearrangement of the protein molecule conformation to expose buried hydrophobic groups is likely to occur.

Improved solubility properties of proteins with modification will make it easier to formulate into different food systems under different conditions.

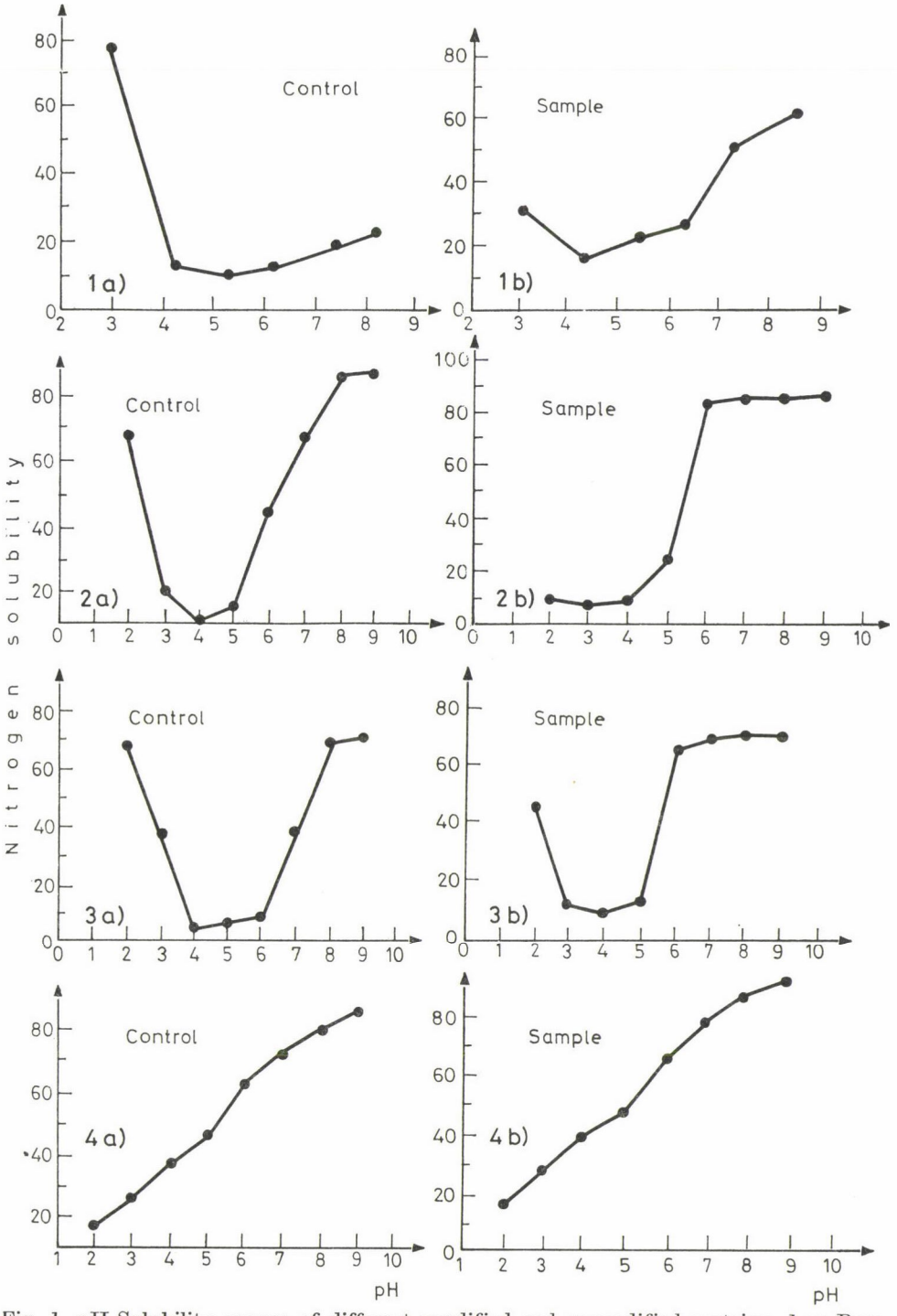

Fig. 1. pH-Solubility curves of different modified and unmodified proteins. 1 a: Pure gluten (b: succinylated 40.8%); 2 a: Pure zein (b: succinylated 67%); 3 a: Commercial zein (b: succinylated 29.6%); 4 a: Egg albumin (b: succinylated 84%)

2.3. Emulsifying capacity of different succinylated proteins

The emulsifying capacity (EC) of different modified protein preparations at pH 7.5 compared with the EC of their unmodified forms are shown in Table 1. The unmodified proteins had a slightly higher emulsifying capacity than those of succinylated ones.

This succinylation made it possible to obtain better solubility without lowering significantly the emulsifying properties of the modified proteins.

Table 1

Effect of succinylation on the emulsifying capacity of different modified proteins (at pH 7.5) compared to their unmodified form (at pH 9.5)

Protein		Percentage of succinylation (%)	Emulsifying capacity (cm^3 oil per g protein)[a]
Gluten	unmodified	0.0	692
	modified	40.8	667
Zein	unmodified	0.0	600
	modified	67.0	625
Zein (commercial)	unmodified	0.0	595
	modified	29.6	465
Egg albumin	unmodified	0.0	725
	modified	84.0	655

[a] Average of three replications, SD: 13.6 $cm^3\ g^{-1}$

2.4. Foaming properties of succinylated protein preparations

The effect of protein concentration on the foam overrun of unmodified proteins at pH 9.5 and succinylated ones at pH 7.5 is shown in Table 2. Increasing the concentration of protein samples led to an increase in foam overrun. The overruns of succinylated proteins were generally higher than those of the unmodified ones especially at 2% protein concentration. This effect of succinylation may be a result of altered protein molecule conformation and facilitated penetration of water molecules as envisaged by KINSELLA and SHETTY (1979).

The greatest change in foam overrun upon protein succinylation was noticed in case of wheat gluten which showed the least value in its unmodified form among the proteins. This might indicate that succinylation has incurred relatively greater changes in gluten molecule conformation than any other pure protein.

Table 2

Effect of protein concentration on the foam overrun of different unmodified and succinylated proteins

Protein	Percentage of succinylation (%)	Protein concentration (%)	Foam overrun[a] (%)	
			Unmodified form (at pH 9.5)	Succinylated form (at pH 7.5)
Gluten	40.8	1	42	42
		2	69	77
		3	67	71
Zein	67.0	1	73	75
		2	75	78
		3	76	79
Zein (commercial)	29.6	1	31	31
		2	33	35
		3	34	36
Egg albumin	84.0	1	78	79
		2	80	82
		3	83	84

[a] Average of three replications; SD: 2.6%

On the other hand egg albumin which had the highest foam overrun value in its unmodified form exhibited the least change in its succinylated form among the pure proteins.

Commercial zein showed the least overrun values either in its unmodified or succinylated form indicating that the lipids and carbohydrate in this product could not only impede the succinylation reaction but also hinder the process of foam formation.

The foam stability of either unmodified or succinylated proteins was generally similar. So, it can be inferred that although succinylation has affected the foam overrun of each protein it has no influence on the stability of that foam.

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COMBINED EFFECTS OF PHYSICAL TREATMENTS AND SPOROSTATIC FACTORS ON *CLOSTRIDIUM SPOROGENES* SPORES

I. COMBINED EFFECTS OF HEAT TREATMENT, NITRITE, REDUCED a_w AND REDUCED pH IN AN ANAEROBIC NUTRIENT MEDIUM

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Preceeding technological studies on combined effects of irradiation, heat and sporostatic agents on *Clostridium sporogenes* in canned luncheon meat, experiments were performed with a model system to study the effects of factors affecting microbiological stability and safety of canned cured meat products preserved by heat treatments milder than the "botulinum cook". Aliquots of OXOID RCM medium were adjusted to NaCl-, nitrite-, and pH-levels similar to those of cured meat products, inoculated with *Clostridium sporogenes* spores and processed at $F_0 = 0.1–0.7$ min heat treatments. Based on the estimation of the number of spores surviving the heat treatment, and the growth during post-process incubation at optimal temperature, the efficacy of various combinations were compared by HAUSCHILD's (1982) method, expressing both spore destruction and post-process inhibitory effects in spore number log-units. The results showed that in the model system used, and presumably in similar mildly-heated cured meat products, the components of curing salt and the pH play significant roles in the microbiological stability. Inhibitory effects were found to be equivalent to 4–7 log-cycles destruction of the test spores.

Keywords: combined effects, *Clostridium sporogenes*, heat, nitrite, a_w, pH

The shelf-stability and microbiological safety of foods are often ensured not by one single antimicrobial agent but by combined action of various microbicidal and/or microbistatic factors. Currently, more and more attention is being devoted to combined effects in food preservation, because combination treatments can often increase the efficacy of food preservation to the extent that a considerable improvement of product quality and significant energy saving can be achieved by appropriate combinations of antimicrobial agents as compared to technologies applying only single effects.

Cooked cured meats are classical examples of traditional preserved foods whose microbiological stability and safety result from the combined action of mild heat treatment, salt content (reduced water activity), nitrite content and pH. Shelf-stable canned cured meats receive generally a much milder heat treatment than that required for a "botulinum cook" adopted in the canning of low acid, high moisture foods. This is possible because the stability of canned cured meats is based not only on heat destruction of microorganisms, but on

synergistic interactions of several factors controlling bacterial spores. Thus, shelf-stable canned cured meats can be preserved by thermal destruction of the vegetative microflora, partial destruction of bacterial spores and subsequent inhibition of the surviving spores by combined effects of salt and nitrite (SILLIKER et al., 1958; GREENBERG et al., 1959; SILLIKER, 1959; PIVNICK et al., 1969).

According to relevant experiments and industrial experience, and provided that bacterial spore levels of the raw materials are rigidly controlled by good manufacturing practice, shelf-stable canned cured meats of pH 6.1–6.7 can be safely manufactured by combinations of brine concentrations of 4–5% NaCl, 120–150 mg sodium nitrite per kilogram and heat dissipation equivalent to F_0 = 0.7–1.5 min. The protection against botulism, afforded by such combinations of destruction and inhibition has been estimated to be equivalent to a 8–9 D heat treatment (HAUSCHILD & SIMONSEN, 1985).

In relation to the above considerations, and for a later comparison with irradiation, it was deemed interesting to investigate combined effects of mild heat treatment, nitrite, reduced a_w and reduced pH on *Clostridium sporogenes* spores inoculated into an anaerobic nutrient medium. Experiments with this model system are reported in the present article. Combined effects of gamma irradiation, heat treatment, reduced a_w and reduced pH on the same spores in canned lucheon meat will be reported in a subsequent paper.

In these studies, the risk analysis method elaborated by Hauschild and co-workers for the quantitative assessment of microbiological safety and stability of cooked cured meat products was applied to compare efficacy of various combination treatments (HAUSCHILD, 1982; HAUSCHILD & SIMONSEN, 1986).

1. Materials and methods

1.1. Test organism and production of spores

Clostridium sporogenes strain V1240 originating from the culture collection of the Microbiology Department of the Central Food Research Institute, Budapest, was used as testorganism.

The stock spore culture of the test-organism in Reinforced Clostridial Medium, RCM (OXOID CM 149) was heat-shocked at 80 °C for 10 min, and subcultured by 3 days of incubation in freshly steamed RCM at 30 °C. After checking for purity and morphological characteristics by phase-contrast microscopy, 2 cm^3 of this subculture were inoculated after the heat-shock into 200 cm^3 RCM and cultured in an anaerobic jar in a nitrogen atmosphere at 30 °C for 1 week. The cell state distribution after incubation was as follows: mature spores,

c. 60%; sporangiospores, 5%; vegetative cells, 35%. The culture was harvested by centrifugation at 23700×g in a refrigerated centrifuge at 5 °C for 25 min and the pellet was washed three times with sterile distilled water and re-centrifuged. The washed cells were finally re-suspended in sterile distilled water and stored in a refrigerator at 0.5 °C until use.

1.2. Investigation of the combined effects of heat treatment, NaCl and nitrite on Clostridium sporogenes *spores*

First experiment. The following media were inoculated with washed spores of *Cl. sporogenes* at 10^4 cm^{-3}, 10^2 cm^{-3} and 5×10^0 cm^{-3} spore concentrations, respectively:

1. RCM containing 3.5% NaCl and 50 mg cm^{-3} nitrite (pH of medium before heat treatment: 6.7);

2. RCM containing 5.5% NaCl and 200 mg cm^{-3} nitrite (pH of medium before heat treatment: 5.8);

Using the crystal liquefaction method of VAS and PROSZT (1955), the water activity in the original RCM medium was less than 0.98, the a_w of media adjusted to 3.5% NaCl level was approx. 0.98, while those containing 5.5% NaCl were estimated a_w between 0.965 and 0.97. These values are in relatively good agreement with the data for a_w versus concentration of pure NaCl solutions of LABUZA (1975).

The first medium represented an a_w (NaCl) level and pH, which may not be sufficiently sporostatic in cured meat products (HAUSCHILD, 1982; HAUSCHILD & SIMONSEN, 1983, 1986), while the second medium was expected to show a significant inhibitory effect on *Clostridium* spores.

At each inoculum level, media variants and heat-treatment levels, 50 replicates of 10 cm^3 volumes were heat treated in an autoclave at $F_0 = 0.5$ and $F_0 = 0.8$ levels, respectively. Heat penetration was determined in a preliminary experiment using an ELLAB thermocouple system.

After heat treatment, a sterilized and molten 1 : 1 mixture of vaseline and paraffin-wax was layered aseptically over each sample to exclude air, and to trap gas bubbles forming during growth of the test-organism. Each set of tubes was incubated at 37 °C for 75 days.

The viable spore counts of the inocula heat-stocked at 80 °C for 10 min, and the number of spores surviving the heat treatment in the inoculated media were estimated by the "most probable number" technique in RCM tubes. Dilution series were prepared with an aqueous solution of 0.1% peptone and 0.9% NaCl.

Second experiment. On the basis of the results of the first experiment the effects of combinations of the following factors were investigated:

pH of media: 6.7 or 5.8 (adjusted by 10% hydrochloric acid); NaCl levels: 0.5%, 3.5%, and 5.5%;
$NaNO_2$ concentrations: 0 mg cm^{-3}; 50 mg cm^{-3}; and 200 mg cm^{-3};
Inoculum levels: 10^6 spores cm^{-3} 10^4 spores cm^{-3}.

Heat treatments: $F_0 = 0$ min, $F_0 = 0.1$ min and $F_0 = 0.7$ min; the latter one in case of the inoculum level of 10^6 cm^{-3}.

Due to the large number of experimental variants, only five replicate samples (test tubes) were investigated. Otherwise, the experimental conditions were similar to those of the first experiment. The experimental batches prepared for incubation without heat treatment were inoculated with heat shocked spore suspensions. Preliminary investigation demonstrated that the spores did not germinate in the RCM medium before the heat treatment of inoculated samples. The incubation period of this experiment was extended to 4 months at 37 °C, to allow for germination of dormant spores (GOULD & HURST, 1969).

1.3. Quantitative assessment of the sporocide and sporostatic efficacy of the combined treatments

In order to estimate comparably the sporocide and sporostatic efficacy of the combined treatments, the *Ds* and *Pr* values were determined according to HAUSCHILD (1982).

The *Ds* values are the lethalities of the physical treatments, calculated as difference in decimal counts (log units) of viable counts of the test organism before and immediately after the physical treatments:

$$Ds = \log N_0 - \log N_t.$$

The *Pr* values express the total protection given by the combined effects. *Pr* is the sum of the decimal destruction (*Ds*) and the decimal inhibition (*In*):

$$Pr = Ds + In.$$

Accordingly, this approach in calculating the efficacy of preservation makes no distinction between destruction and inhibition. The *Pr* values can be calculated from the spoilage ratio of the inoculated experimental batches as

$$Pr = \log 1/P,$$

where P is the probability of individual spores to survive the physical treatments, and to overcome the inhibition by sporostatic factors. P is estimated as

$$P = MPN/s,$$

where *MPN* is the most probable number of spores capable of growth per experimental, unit, and

s is the number of spores of the test organism per experimental unit.

The *MPN* value is calculated from the spoilage (growth) data according to Halvorson and Ziegler's equation:

$$MPN = \ln (n/q),$$

where n is the total number of experimental units in the experimental batch,
q is the number of non-spoiled units.

2. Results and discussion

2.1. First experiment

The viable cell counts of cultures immediately before and after the heat treatments are given in Table 1.

Table 1

Destruction of Cl. sporogenes *spores by heat treatments in media variants of the first experiment*

Media variant	pH		Heat treatment F_0 (min)	*MPN* cm^{-3}	
	before heat treatment	after heat treatment		before heat treatment	after heat treatment
RCM containing NaCl 3.5% and nitrite 50 mg dm^{-3}	6.7	6.7	0.5	9×10^3	7×10^0
			0.8	9×10^3	7×10^0
			0.5	1×10^2	$<2\times10^0$
			0.8	1×10^2	$<2\times10^0$
RCM containing NaCl 5.5% and nitrite 200 mg dm^{-3}	5.8	5.7	0.5	9×10^3	9×10^0
			0.8	9×10^3	4×10^0
			0.5	1×10^2	$<2\times10^0$
			0.8	1×10^2	$<2\times10^0$

In both media-variants, inoculated to approx. 10^4 cm^{-3} level, heat treatments of $F_0 = 0.5$–0.8 min resulted in a lethality of approximately three log-cycles of viable spore counts. In experimental batches of 1×10^2 cm^{-3} or 5×10^0 cm^{-3} inoculum levels, the number of survivors in the heated samples were below the detection limit; i.e. less than 2 cfu cm^{-3}. Therefore, data related to the lowest inoculum level are not given in Table 1.

From the reduction of viable counts at $F_0 = 0.5$–0.8 min heat treatments a D_{121}o-value of 0.16–0.26 min can be estimated. In the review of INGRAM (1969) D_{120}-values of 0.1–1.5 minutes are given from a diverse literature for spores of *Clostridium sporogenes*, and a D-value of 1.3 min at 121 °C for *Cl. sporogenes* PA 3679 in particular, all in phosphate buffer, assuming ex-

ponential heat destruction kinetics. Therefore, our strain seems to be less heat resistant than strain PA 3679 but falls into the above range of D-values reported by Ingram.

None of the tubes of the first experiment showed growth during the 75 days of incubation at 37 °C, thus, both media-variants ensured inhibition of spores which survived the heat treatments.

According to literature data (ICMSF, 1980), pH = 5.0 is the minimal pH for growth of *Clostridium sporogenes*, and the inhibitory levels of nitrite are in the range of 80–200 mg cm^{-3} at pH 6.0, and in the range of 320–1280 mg cm^{-3} at pH = 7.0. The inhibitory concentration of NaCl was reported as 8.7–9.5% (ICMSF, 1980). The fact that spores surviving the heat treatment could not grow in our experiment even in the medium, which contained considerably less NaCl and nitrite than the above data, points to a combined effect or even synergistic interaction of factors on heat-damage spores.

It has been shown by PERIGO and co-authors (1967) that nitrite heated in a bacteriological medium was more inhibitory towards growth of *Cl. sporogenes* than nitrite added aseptically to the medium after autoclaving. PERIGO and ROBERTS (1968) showed an enhanced inhibitory effect of nitrite heated in laboratory media against 30 strains of clostridia. Therefore, the formation of an unidentified "Perigo factor" may explain the strong growth-inhibition observed in our model system. Formation of nitrosothiols or related compounds, whose inhibitory effect was demonstrated among others by Hungarian workers (INCZE et al., 1974) may play a role in the above system. The topic has been reviewed recently by WOODS and co-workers (1989).

2.2. Second experiment

The direct effect of heat treatments on the viable cell counts of inoculated batches, and the results of incubation are summarized in Tables 2 and 3. All but one positive sample developed signs of growth within 2 months of incubation.

The mild heat treatment $F_0 = 0.1$ resulted in approx. 0.5, and the $F_0 = 0.7$ treatment in 3.4 log-cycles reducing in numbers of viable spores, i.e.

Table 2

Destruction of Cl. sporogenes *spores by heat treatments in the second experiment*

Heat treatment F_0 (min)	*MPN* cm^{-3} (log *MPN*)	Heat destruction in log cycles (*Ds*-values)
0	9×10^5	—
0.1	3×10^5	0.47
0.7	4×10^2	3.35

Table 3

Combined effects of heat treatment, NaCl (reduced a_w), $NaNO_2$ and reduced pH on growth of Clostridium sporogenes *spores inoculated into RCM*[a]

Heat treatment F_0 (min)	$NaNO_2$ (mg dm^{-3})	Initial count: 9×10^5 cm^{-3}						Initial count: 9×10^3 cm^{-3}					
		pH = 6.7			pH = 5.8			pH = 6.7			pH = 5.8		
		0.5% NaCl[b]	3.5% NaCl	5.5% NaCl	0.5% NaCl	3.5% NaCl	5.5% NaCl	0.5% NaCl	3.5% NaCl	5.5% NaCl	0.5% NaCl	3.5% NaCl	5.5% NaCl
	0	5	5	5	5	5	5	5	5	5	5	5	5
0	50	5	5	5	0	0	0	5	5	5	0	0	0
	200	5	5	2	0	0	0	5	0	0	0	0	0
	0	5	5	5	5	5	1	5	5	5	5	4	0
0.1	50	5	5	0	0	0	0	5	5	0	0	0	0
	200	4	0	0	0	0	0	0	0	0	0	0	0
	0	5	5	5	5	3	0						
0.7	50	0	0	0	0	0	0						
	200	0	0	0	0	0	0						

[a] Number of positive samples from 5 replicates after 125 days of incubation at 37 °C
[b] 0.5% NaCl: $a_w > 0.98$; 3.5% NaCl: $a_w = 0.98$; 5.5% NaCl: $a_w = 0.965 - 0.97$

the heat destruction kinetics of *Clostridium sporogenes* spores followed exponential kinetics. From these values the D_{121}o-value of our strain was estimated to be approx. D = 0.21 min, which is very similar to the heat resistance of spores of the most resistant A and B types of *Clostridium botulinum* (STUMBO, 1963).

Table 3 shows manyfold interactions of the antimicrobial factors in our model system. It can be seen that there was a strong pH-effect in the nitrite containing samples, and at given pH-levels, increasing heat treatments enhanced increasingly the sensitivity of survivors for nitrite and NaCl.

On the basis of Table 3, the efficacy of various combinations was calculated following the method of HAUSCHILD (1982) and HAUSCHILD and SIMONSEN (1985), estimating the "decimal protection" (*Pr*), "decimal destruction" (*Ds*) and "decimal inhibition" (*In*)-values, in D-units as described in Chapter 1.3.

Due to the *MPN*-concept of this estimation, the quantification could be made for those five treatment-variants, where both positive and negative tubes (sample units) were found at the end of the incubation. The increasing order of total antimicrobial efficacy of these combinations expressed in *Pr*-values is given in Table 4.

The order of combinations according to the increase of the sporostatic component (*In*-value) of the total antimicrobial effect can be seen in Table 5.

Table 4

Decimal protection (Pr) *values*

Combined factors:				*Pr*-values:
$F_0 = 0.1$;	NaCl = 3.5%;	pH = 5.8;	$NaNO_2$ = 0 ppm	4.75
$F_0 = 0.1$;	NaCl = 0.5%;	pH = 6.7;	$NaNO_2$ = 200 ppm	6.75
$F_0 = 0.7$;	NaCl = 3.5%;	pH = 5.8;	$NaNO_2$ = 0 ppm	7.00
$F_0 = 0$;	NaCl = 5.5%;	pH = 6.7;	$NaNO_2$ = 200 ppm	7.25
$F_0 = 0.1$;	NaCl = 5.5%;	pH = 5.8;	$NaNO_2$ = 0 ppm	7.62

Table 5

Decimal inhibition (*In*) *values*

Combined factors:				*In*-values:
$F_0 = 0.7$;	NaCl = 3.5%;	pH = 5.8;	$NaNO_2$ = 0 ppm	3.65
$F_0 = 0.1$;	NaCl = 3.5%;	pH = 5.8;	$NaNO_2$ = 0 ppm	4.28
$F_0 = 0.1$;	NaCl = 0.5%;	pH = 6.7;	$NaNO_2$ = 200 ppm	6.28
$F_0 = 0.1$;	NaCl = 5.5%;	pH = 5.8;	$NaNO_2$ = 0 ppm	7.15
$F_0 = 0.1$;	NaCl = 5.5%;	pH = 6.7;	$NaNO_2$ = 200 ppm	7.25

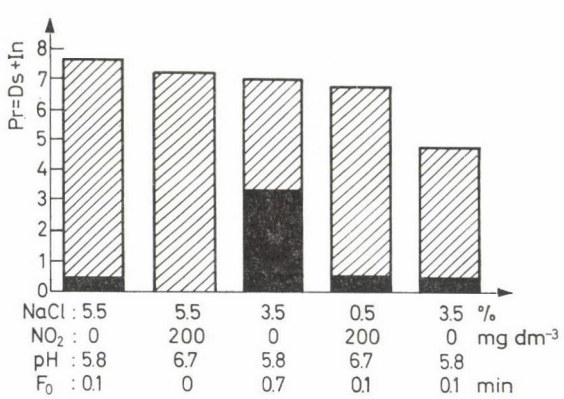

Fig. 1. Efficacy of combination of heat treatment. NaCl (reduced a_w), $NaNO_2$ and reduced pH on destruction and inhibition of spores of *Clostridium sporogenes* inoculated into RCM. Pr: total protection; ▩: spore destruction (*Ds*); ▨: inhibition (*In*)

Figure 1 shows both the decimal destruction and decimal inhibition components of the total antimicrobial efficacy of these combinations.

The results of these experiments and the estimated efficacy values show that curing salts and suboptimal pH play a significant role in the microbial

stability of mildly heat-treated model system, and presumably also in that of pasteurized shelf-stable cooked cured meat products. Depending on the actual combinations, these factors resulted in growth inhibition (*In*-values) which was equivalent to approx. 4 to 7 log-cycles of heat destruction of the test organism, i.e. equivalent to a heat-processing range of F_0 = 0.8–1.5 min.

On the basis of these preliminary experiments, it can be concluded that our test organism can be profitably applied in studying combined preservation methods, and that the Hauschild-approach lends itself to quantification of the efficacy of combined treatments. Such experiments with canned luncheon meat will be reported in the subsequent paper (FARKAS et al., 1991).

*

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COMBINED EFFECTS OF PHYSICAL TREATMENTS AND SPOROSTATIC FACTORS ON *CLOSTRIDIUM SPOROGENES* SPORES

II. COMBINED EFFECTS OF GAMMA RADIATION, HEAT TREATMENT, REDUCED a_w AND REDUCED pH IN CANNED LUNCHEON MEAT

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In three experimental series luncheon meat mixtures containing a brine of 3.8–4.0% NaCl and $NaNO_2$ of 33–48 mg per kg, and heavily inoculated with *Clostridium sporogenes* spores were treated with gamma radiation doses of 3–4 kGy, and a subsequent heat treatment of $F_0 = 0.5$–0.86 min. In order to increase inhibitory effects on surviving spores, the pH of the meat paste was reduced from pH = 6.2–6.5 to pH = 5.5–5.7 by the addition of lactic acid or ascorbic acid, and the water activity of the paste was slightly reduced from its initial value of 0.95–0.96 by the addition of a salt-free enzymatic hydrolysate of soy protein, or potassium chloride or K-lactate prior to processing.

Doses of irradiation of 3–4 kGy had little lethal effect on spores of *Clostridium sporogenes*, while the same radiation treatment combined with above heat treatment synergistically controlled this important spoilage organism, particularly in combination with a reduced pH of the product. However, the acidulants and/or humectants applied adversely affected the sensory parameters. Thiamine retention was lower after the combined treatment than after traditional commercial sterilization. Therefore, further research is necessary to establish microbiologically efficient combinations which affect the thiamine content and sensory quality of the product to a lesser extent.

Keywords: combined effects, *Clostridium sporogenes*, gamma radiation, heat, a_w, pH, luncheon meat

Due to concerns about the health impact of the use of high nitrite and salt contents of meat products, there is a tendency to reduce salt and nitrite levels of meat products. However, reduced nitrite and salt input has to be compensated by other factors assuring microbiological safety, such as more intense thermoprocess (PIVNICK et al., 1969, PRÄNDL, 1980). In Hungary, where brine concentrations of no more than 4%, and nitrite levels of less than 50 mg per kg are used, canned luncheon meats are processed presently as regular canned fooods, applying F_0 values of up to 6.5 min. However, the quality of the product and the energy consumption of the process are adversely affected by applying such a severe heat treatment.

In a previous paper (FARKAS & ANDRÁSSY, 1991) preliminary experiments on combined effects of mild heat treatment, nitrite, reduced a_w and reduced pH on *Clostridium sporogenes* spores in a model system have been

reported. The aim of the experiments described in the present paper was to estimate, to what extent reduced salt- and nitrite levels and a reduced heat treatment of canned luncheon meat can be compensated by 1) a radiation treatment and/or 2) slightly reduced pH and water activity, using additives which do not increase the sodium content of the product.

1. Materials and methods

1.1. Test organism

The *Clostridium sporogenes* strain and production of its spores have been described in the previous article (FARKAS & ANDRÁSSY, 1991). Spores produced in RCM medium (OXOID CM 149) were harvested by centrifugation, then washed and treated in 0.1 mg cm^{-3} aqueous lysozyme solution for overnight in a refrigerator. Lysozyme-treated spores were cleaned from lysozyme and cell debris by 10 cycles of repeated centrifugation and aqueous resuspension. In preliminary experiments reported elsewhere (FARKAS, 1987) $D_{121} = 0.2$ min was estimated for heat resistance, and a $D_{exp} = 2$ kGy was established for radiation resistance in aqueous suspension with a survival curve showing a shoulder part extending to approx. 2–5 kGy dose. Thus, both the heat resistance and the radiation resistance of these putrefactive spores are similar to those of proteolytic strains of *Clostridium botulinum* A and B, as given in the literature (ROBERTS & INGRAM, 1965; ANELLIS et al., 1967; STUMBO, 1973).

1.2. Test materials and experimental series

Luncheon meat mixtures were prepared in the pilot plant of the Hungarian Meat Research Institute, Budapest. Unless otherwise stated, the basic composition of the meat mixture corresponded to Hungarian industrial standards.

After mixing the meat components with additives, and curing the mixture at 4 °C for one day, the luncheon meat paste was prepared in a mincing machine using an extrusion plate of 5 mm perforations.

Approximately 70 g of meat mixture were stuffed into cans of 1/10 size and each can was inoculated with spores of *Clostridium sporogenes*. The experimental batches consisted of 20–50 cans per treatment.

Three experimental series were prepared:

Luncheon meat units containing brine (% salt in the aqueous phase) of 3.8% and $NaNO_2$ of 33 mg per kg were inoculated with 3×10^5 spores per can, (confidence interval: $1\times10^5 - 1.2\times10^6$) sealed and treated with combinations of $F_0 = 0.54 \pm 0.1$ min and a gamma radiation dose of 4 kGy.

According to our preliminary tests, this is approximately the maximum dose which can be applied to this product without adversely affecting its flavour. The role of the sequence of the two physical treatments has been also investigated in this experiment. The water activity and pH of the canned product were 0.96 and 6.5, respectively.

In the second experimental series the brine concentration was 4.0% and the nitrite input amounted to 48 mg per kg. Half of the experimental batches was supplemented with 5% soy protein hydrolysate and lactic acid was added. The latter resulted in a pH-drop from pH 6.2 to pH 5.7 MOL and TIMMERS (1970) reported that a similar pH-reduction by incorporation of glucono-delta-lactone into comminuted meat products improved the stability of the pasteurised products considerably.

The soy protein hydrolysate was prepared by treatment with an Alcalase enzyme preparation at the Technology Department of the Central Food Research Institute, in a similar way as described by VALLEJO–CORDOBA and co-workers (1986). The water activity of the luncheon meat supplemented with the protein hydrolysate was $a_w = 0.945$ as compared to 0.95 of the product produced without the hydrolysate addition. The inoculum level was increased to 3×10^6 spores per can in this experimental series, and in the knowledge of the synergistic effect of pre-irradiation and subsequent heat treatment, the combination of 4 kGy followed by a heat treatment of $F_0 = 0.8$, was applied.

In the third experimental series, utilization of ascorbic acid (AsH) as acidulant for pH-reduction, and KCl or K-lactate for a slight a_w-reduction was investigated. The potassium salts were chosen in order to avoid an increase of the sodium content of the product. The luncheon meat paste was divided into four 6 kg parts, and the following amounts of experimental additives were mixed with them:

Batch one: none
Batch two: 44.5 g ascorbic acid (to reduce the pH from 6.0 to 5.4)
Batch three: 1.5% KCl
Batch four: 2.0% potassium lactate

The final paste has been analysed for gross composition in the Hungarian Meat Research Institute, with the following results: moisture 61.8% (w/w); raw protein 20.4%; fat 14.6%; NaCl 3.0%; $NaNO_2$ 83.7 mg per kg; glucose 0.94%.

The anaerobic colony count of the uninoculated paste proved to be approx. 10^4 per can. Cans containing approx. 70 g amounts of meat paste were inoculated with approx. 6.4×10^6 spores of *Clostridium sporogenes* per unit before closing. Uninoculated cans were also kept from each batch for sensory testing and vitamin analysis subsequent to combination treatments.

In view of the results of the first two experimental series, the sequence irradiation → heat treatment was applied only.

The heat treatment, which had been designed to represent the traditional canning process of $F_0 \sim 6.0$ proved to be $F_0 = 5.6$ min on the average, with a standard deviation of 1.1 min, while the mild heat processing aiming at $F_0 \sim 0.8$ was found to be $F_0 = 0.86$ on the average, with a standard deviation of 0.13 min.

The various treatments of experimental batches in the third experimental series are summarized in Table 1.

Table 1

Treatments applied in the third experimental series

Treatment code	Treatment
1	$F_0 = 5.6$
2	$F_0 = 0.86$
23	3 kGy + $F_0 = 0.86$
3	ascorbic acid (AsH) + $F_0 = 0.86$
33	ascorbic acid (AsH) + 3 kGy + $F_0 = 0.86$
4	1.5% KCl + $F_0 = 0.86$
43	1.5% KCl + 3 kGy + $F_0 = 0.86$
5	2.0% Potassium lactate + $F_0 = 0.86$
53	2.0% Potassium lactate + 3 kGy + $F_0 = 0.86$

1.3. Radiation treatment

Radiation treatment of spore suspensions or canned product was performed at ambient temperature by a self-shielded Co-60 gamma irradiator of type RH-gamma-30 in the Central Food Research Institute. The dose rate was 6.4 kGy h^{-1}.

1.4. Heat treatment

The cans were heated in a Stock Pilot Rotor 900 autoclave in steam at 105 °C. The thermal process was monitored by distributing cans of luncheon meat fitted with thermocouples throughout the load. F_0 values were calculated during the heat treatment by a Thermocomp computer, assuming a z value of 10 °C, and the heating was interrupted at prescribed F_0 values by rapid cooling in water.

1.5. Estimation of viable spores

Cans were opened aseptically and viable spore counts were estimated before and immediately after treatments, by the most probable number technique, inoculating 5–50 replicates into RCM tubes at each dilution level. The inoculated tubes were incubated anaerobically at 37 °C for 14 days, and tubes giving evidence of growth were recorded.

1.6. pH- and a_w*-measurements*

After the heat treatment, the water activity values of the products were estimated by a Novasina a_w-tester at 25 °C, and pH values of both the solid phase and the exudated liquid were measured by an electrical pH-meter.

1.7. Incubation and examination of cans for spoilage

Cans were incubated at 37 °C and examined for swelling at weekly intervals during the first 2 months. After 3.5 months of incubation, all cans were examined for other symptoms of spoilage.

1.8. Quantitative assessment of the sporocidal and sporostatic efficacy of the combined treatments

In order to estimate the sporocidal and sporostatic efficacy of the combined treatments, the *Ds* and *Pr* values were determined according to HAUSCHILD's method (1982).

The *Ds* values are the lethalities of the physical treatments, calculated as differences in log units of viable counts of the test organism before and immediately after the physical treatments:

$$Ds = \log N_0 - \log N_t$$

The *Pr* values express the total protection given by the combined effects. *Pr* is the sum of destruction (*Ds*) and inhibition (*In*):

$$Pr = Ds + In$$

Accordingly, this approach in calculating the efficacy of preservation makes no distinction between destruction and inhibition.

The *Pr* values can be calculated from the spoilage rate of the inoculated experimental batches as

$$Pr = \log 1/P$$

where P is the probability of individual spores to survive the physical treatments, and to overcome the inhibition exerted by sporostatic factors. The

calculation of P-value is described in our previous paper (FARKAS & ANDRÁSSY, 1991).

Because $1/P$ expresses the number of spores effectively controlled, in the knowledge of the "natural" contamination of a commercial product with the critical microorganism, and assuming its equivalence with the test organism in the inoculated pack studies, it is possible to estimate the number of commercial cans which would be effectively protected using the treatment of the inoculated pack. This number is $1/(P \times i)$, where i is the "natural" incidence of the critical microorganism per can (HAUSCHILD, 1982).

1.9. Quality assessment of non-spoiled samples

Samples taken immediately after the treatments and non-spoiled samples after storage were analysed as follows:

1.9.1. Measurement of juice release and consistency of canned samples. In order to estimate the water binding capacity of canned samples as affected by physical and chemical factors involved in various combination treatments, cans were put into warm water of 40 °C for a short while then opened and the released liquid phase estimated volumetrically after the liquid separated into aqueous and fat phases.

The consistency of the solid phase was measured by a Dispersitron penetrometer at ambient temperature. The softness of the samples was expressed in arbitrary units proportional to the depth of penetration of the plunger.

1.9.2. Colour measurement. The colour of the samples was objectively measured with a Momcolor-D tristimulus colorimeter by the reflectance technique as described by URBÁNYI and HORTI (1989) and the colour stimulus characteristics were established in the CIELAB system. Nine measurements were made per sample.

1.9.3. Sensory testing of uninoculated samples. A non-parametric sensory testing method was performed by 9 panelists, marking their respective scores for taste, smell (flavour) and consistency of the samples on 10 cm long straight lines, where the end-points represented the completely unacceptable and the excellent qualities, respectively. The distances of the marks from the starting point of the line were evaluated by analysis of variance, considering that the total length was equal with a 5-unit sensory scale.

1.9.4. Estimation of thiamine content. Thiamine (vitamin B_1) is sensitive to both heat and radiation. Therefore, tentative studies were performed to estimate the thiamine content of combined-treated samples as compared to that of heat-treated samples. Thiamine contents were determined in the National Institute of Nutrition and Food Hygiene, Budapest, according to the standard microbiological method of the Scientific Society of the Hungar-

ian Food Industry (M.É.T.E., 1970). A strain of *Lactobacillus fermenti* served as test organism and its growth in Bacto Thiamine Assay Medium (DIFCO) was evaluated turbidimetrically after 16 hours incubation at 37 °C.

2. Results

2.1. First and second experimental series

The spoilage percentage of inoculated cans at the end of the incubation test are summarized in Figs 1 and 2 for the first and the second experimental series, respectively.

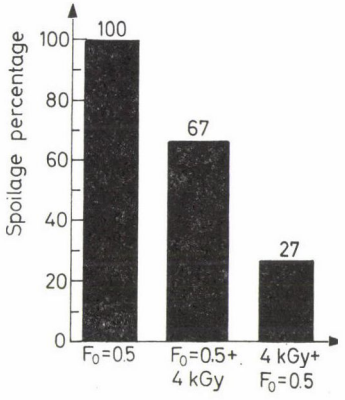

Fig. 1. Spoilage percentage of experimental batches of canned luncheon meat inoculated with 3×10^5 *Cl. sporogenes* spores per can. NaCl (% brine) = 3.8; $NaNO_2$ = 33 mg per kg. a_w = 0.96; pH = 6.5

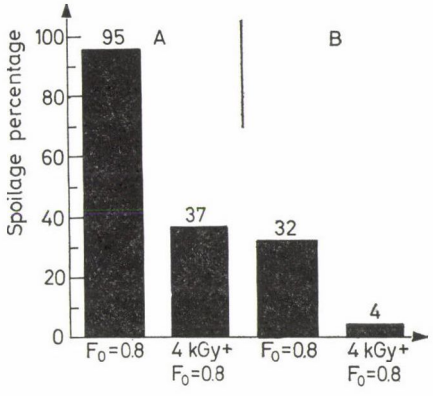

Fig. 2. Spoilage percentage of experimental batches of canned luncheon meat inoculated with 3×10^6 *Cl. sporogenes* spores per can. NaCl (% brine) = 4.0; $NaNO_2$ = 48 mg per kg. A: a_w = 0.95; pH = 6.2. B: a_w = 0.945 (soy hydrolysate); pH = 5.7 (lactic acid)

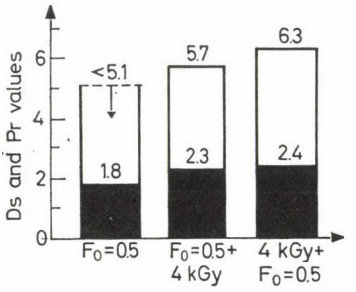

Fig. 3. Efficacy of the combination treatmenrs of canned luncheon meat expressed in lethalities (*Ds* values) and total protection (*Pr* values) given by the treatments. Inoculum: 3×10^5 *Cl. sporogenes* spores per can. NaCl (% brine) = 3.8; $NaNO_2$ = 33 mg per kg. a_w = 0.96; pH = 6.5. ▩: *Ds*; ▩□: *Pr* – values

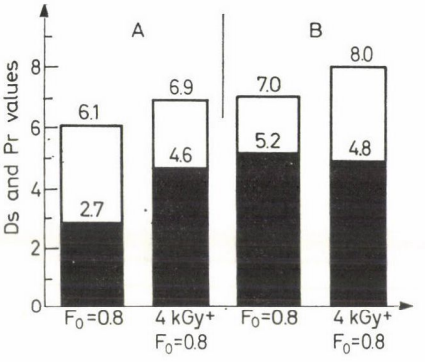

Fig. 4. Efficacy of combination treatments of canned luncheon meat as expressed by lethalities (*Ds* values) and total protection (*Pr* values) given by the treatments. Inoculum: 3×10^6 *Cl. sporogenes* spores per can. NaCl (% brine) = 4.0; $NaNO_2$ = 48 mg per kg. A: a_w = 0.95; pH = 6.2. B: a_w = 0.945 (soy hydrolysate); pH = 5.7 (lactic acid). For symbols see Fig. 3

Figures 3 and 4 demonstrate that the slight decrease of the water activity and the drop of the pH in the canned lucheon meat by the use of protein hydrolysate and lactic acid addition considerably increased the efficacy of both the heat treatment and that of the combination of the 4 kGy dose and the heat treatment of F_0 = 0.8 min.

Figure 4 shows that the increased efficacy is related to the increased *Ds* value, rather than the *In* value. This makes it probable that the pH-drop was the dominant factor in the increased efficacy of the combination treatment, since the slight reduction of the a_w from 0.95 to 0.945 could not considerably influence the heat and radiation resistance of the spores.

Assuming a contamination level of the commercial product with one hundred putrefactive clostridial spores per can (STEINKRAUS & AYRES, 1964), we would obtain numbers of "safe" cans (see Chapter 1.8) for uninoculated experimental materials as they are given in Fig. 5.

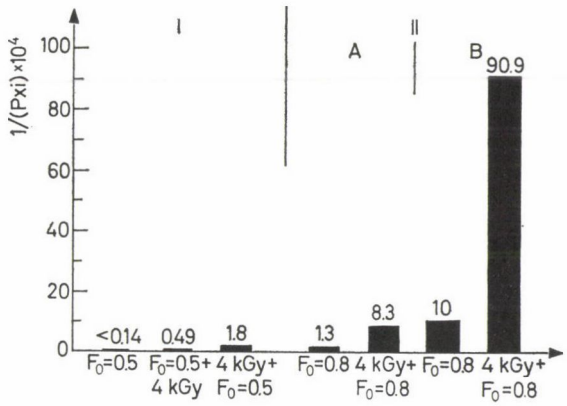

Fig. 5. Relative numbers of commercial cans as a function of treatments if the commercial product carries one hundred sporogenes spore per can ($1/P$ relates to the number of spores effectively controlled; i = incidence of "native" spores per can). I: First series: NaCl (% brine) = 3.8; $NaNO_2$ = 33 mg per kg, a_w = 0.96; pH = 6.5. II: Second series: NaCl (% brine) = 4.0; $NaNO_2$ = 48 mg per kg. A: a_w = 0.95; pH = 6.2 B: a_w = 0.945 (soy hydrolysate); pH = 5.7 (lactic acid)

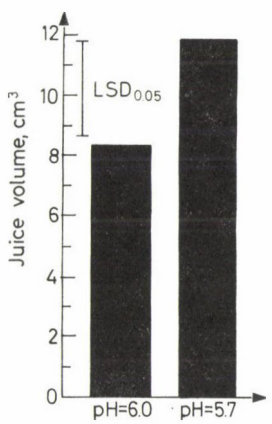

Fig. 6. Average released juice volumes per can in the second series as affected by the pH of the product. $LSD_{0.05}$ = least significant difference at $\alpha \leq 0.05$ probability of error

Figures 6 and 7 show the released juice volume and the consistency of the experimental luncheon meat samples from the second experimental series as a function of the pH.

It can be seen that the released juice volume per experimental can was significantly increased while the consistency of the canned product was significantly hardened by a reduction of the pH from 6.2 to 5.7. This adverse effect is related to a reduced water holding capacity of proteins at reduced pH, particularly, if they are heat treated.

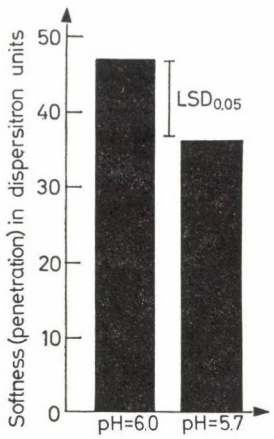

Fig. 7. Average softness values in canned luncheon meat as affected by the pH of the product. $LSD_{0.05} \leq$ least significant difference at $\alpha \leq 0.05$ probability of error

2.2. *Third experimental series*

2.2.1. Changes in pH and a_w *changes as an effect of additives.* The pH- and a_w-values of the canned luncheon meat as affected by various treatments and additives are shown in Table 2.

As compared to the original pH (5.96) of the unheated paste and to the pH of the unheated paste acidified by ascorbic acid (pH 5.4), respectively, a pH-increase of 0.2–0.3 was noted as a result of the heat treatment. The water activity was only slightly reduced (from 0.9 to 0.95) by supplementing the paste with 1.5% KCl or 2.0% potassium-lactate.

Table 2

pH and a_w *of experimental batches as affected by additives*

Code	Treatment	pH after cooling		a_w
		solid phase	liquid phase	
1[a]	$F_0 = 5.6 \pm 1.1$	6.09	6.32	0.96
2	$F_0 = 0.86 \pm 0.13$	6.17	6.30	0.96
23	3 kGy + $F_0 = 0.86$	6.17	6.30	0.96
3	AsH + $F_0 = 0.86$	5.61	5.71	0.96
33	AsH + 3 kGy + $F_0 = 0.86$	5.54	5.65	0.96
4	1.5% KCl + $F_0 = 0.86$	6.21	6.25	0.95
43	1.5% KCl + 3 kGy + $F_0 = 0.86$	6.20	6.24	0.95
5	2.0% Potassium lactate + $F_0 = 0.86$	6.17	6.19	0.95
53	2.0% Potassium lactate + 3 kGy + $F_0 = 0.86$	6.17	6.18	0.95

[a] Uninoculated batch only
AsH = extra addition of ascorbic acid after curing, as described in 1.2

2.2.2. Effect of various treatments on the viable count of anaerobic spores in luncheon meat samples. The log cfu-values per can before and after various treatments are compared in Table 3.

It can be seen that the 3 kGy irradiation dose did not significantly reduce the cfu count of anaerobic spores in various luncheon meat samples. The $F_0 = 0.86$ heat treatment singly resulted in one-and half log cycles reduction in the paste of regular pH, while approx. two log cycles reduction

Table 3

Colony count of anaerobic spores in luncheon meat inoculated with 6.4×10^6 Clostridium sporogenes *spores per can, as affected by various treatments*

Samples	$\log_{10}$ CFU per can	
	average	95% confidence intervals
Unheated paste	6.56[a]	6.12–6.99
Unheated paste, irradiated at 3 kGy	5.91	5.50–6.48
$F_0 = 0.86$	5.00	4.52–5.60
3 kGy + $F_0 = 0.86$	2.76	2.37–3.19
Unheated paste, supplemented with ascorbic acid	6.66	6.24–7.13
Unheated paste, supplemented with ascorbic acid and irradiated (3 kGy)	6.66	6.24–7.13
Ascorbic acid-supplemented and heated ($F_0 = 0.86$)	4.64	4.23–5.12
Ascorbic acid supplemented + 3 kGy + $F_0 = 0.86$	1.83	1.20–2.57
Unheated paste, supplemented with KCl	6.88	6.54–7.30
Unheated, KCl-supplemented + 3 kGy	6.63	6.22–7.11
KCl + $F_0 = 0.86$	5.23	4.84–5.77
KCl + 3 kGy + $F_0 = 0.86$	4.65	4.23–5.13
Unheated, Potassium lactate supplemented	6.46	6.04–6.96
Unheated, Potassium lactate supplemented + 3 kGy	6.88	6.54–7.30
Potassium lactate + $F_0 = 0.86$	5.45	5.02–5.94
Potassium lactate + 3 kGy + $F_0 = 0.86$	4.66	4.24–5.13
[a] Anaerobic count in unheated, uninoculated sample:	4.04	3.56–4.64

occurred in the presence of ascorbic acid which decreased the pH to 5.4. In the presence of additives, which slightly reduced the a_w, some tendency of better survival of heat-treated spores can be noted, however, the differences are not statistically significant. Combination of irradiation and the heat treatment synergistically reduced the colony count of the spores. This enhanced heat destruction by preliminary irradiation was much more pronounced in those batches, where the heat treatment was more intensive.

2.2.3. Effect of various treatments on the microbial stability of canned luncheon meat inoculated with Clostridium sporogenes *spores.* The proportion

of non-swollen cans of experimental batches after 8 weeks and 12 weeks incubation, resp., are shown in Table 4.

It is shown in Table 4 that $F_0 = 0.86$ alone or combination with a preirradiation dose of 3 kGy failed to prevent spoilage of severely inoculated luncheon meat. Supplementing the combination treatment with the addition of KCl or potassium lactate, which reduced slightly the water activity, delayed spoilage, in spite of the fact that spore destruction by the combined treatment was much lower in the presence than in the absence of these additives (see

Table 4

Effect of various treatments on the microbial stability of canned luncheon meat inoculated with Clostridium sporogenes *spores*
(Incubation temperature: 37 °C)

Experimental batches	Proportion of non-swollen cans after	
	8 weeks	12 weeks
$F_0 = 0.86$	0/20	0/20
3 kGy + $F_0 = 0.86$	0/20	0/20
AsH + $F_0 = 0.86$	16/20	15/20
AsH + 3 kGy + $F_0 = 0.86$	20/20	20/20
KCl + $F_0 = 0.86$	0/20	0/20
KCl + 3 kGy + $F_0 = 0.86$	10/20	0/20
Potassium lactate + $F_0 = 0.86$	1/20	0/20
Potassium lactate + 3 kGy + $F_0 = 0.86$	6/20	0/20

For key to abbreviations, see legend to Table 1

Table 3). Obviously, in samples of slightly reduced a_w, the sporostatic effect was higher than in samples without these additives. This inhibition, however, could not prevent long term spoilage at the optimum growth temperature of the test organism. Reducing the pH of luncheon meat by ascorbic acid which enhanced the synergistic effect of the combination treatment, provided also a significant inhibition of spores surviving the treatment of $F_0 = 0.86$, and resulted in a shelf-stable product, when this mild heat treatment was combined with a previous irradiation at 3 kGy.

2.2.4. Quantitative estimation of the sporocidal and sporostatic efficacy of various treatments. On the basis of results summarized in Tables 3 and 4, the lethality (*Ds*-value) and the total protection (*Pr*-value) provided by the combination of the sporocidal and sporostatic effect have been estimated. The results are given in Table 5.

Table 5 shows that the pH-reduction by ascorbic acid increased the *Pr*-value of the mildy heat treated luncheon meat by more than one unit i. e. the number of shelf-stable cans would be more than 10 times higher. Combining

Table 5

Quantification of sporocidal and sporostatic effects of various treatments according to HAUSCHILD's *method (1982) of evaluation*

Treatment	Ds	Pr	In
$F_0 = 0.86$	1.56	< 6.32	< 4.76
3 kGy + $F_0 = 0.86$	3.80	< 6.32	< 3.52
AsH + $F_0 = 0.86$	2.02	7.35	5.33
AsH + 3 kGy + $F_0 = 0.86$	4.83	8.10	3.27
KCl + $F_0 = 0.86$	1.65	< 6.32	< 4.67
KCl + 3 kGy + $F_0 = 0.86$	2.23	< 6.32	< 4.09
Potassium lactate + $F_0 = 0.86$	1.01	< 6.32	< 5.31
Potassium lactate + 3 kGy + $F_0 = 0.86$	1.80	< 6.32	< 4.52

For key to abbreviations, see Table 1 and Chapter 1.8.

Table 6

Juice- and fat-release in canned luncheon meat as affected by various treatments
(Average values of 4—6 replicate cans per treatment)

Code	Treatment	Juice release (cm^3 per can)		Fat release (cm^3 per can)	
1	$F_0 = 5.6$	13.6[a]	ab	2.6	ab
2	$F_0 = 0.86$	12.9	a	2.5	a
23	3 kGy + $F_0 = 0.86$	13.6	ab	2.3	a
3	AsH + $F_0 = 0.86$	17.6	d	2.75	abc
33	AsH + 3 kGy + $F_0 = 0.86$	19.0	e	3.4	cd
4	1.5% KCl + $F_0 = 0.86$	14.5	bc	3.25	bcd
43	1.5% KCl + 3 kGy + $F_0 = 0.86$	14.75	c	3.5	d
5	2.0% Potassium lactate + $F_0 = 0.86$	15.4	e	3.25	bcd
53	2.0% Potassium lactate + 3 kGy + $F_0 = 0.86$	17.3	d	4.4	e

[a] Means not followed by the same letter in the same column are significantly different from each other ($\alpha \leq 0.05$)
For key of abbreviations, see Table 1.

pH-reduction with the synergistic combination treatment of 3 kGy + F_0 = = 0.86, a significant further increase of spore destruction and bacteriological stability was observed.

2.2.5. Juice- and fat-release as affected by the combination treatments. Table 6 shows the volumes of aqueous exudates and the fat phase found in replicate samples.

It can be seen from Table 6 that the reduction of the pH by ascorbic acid to pH 5.4–5.7 or the addition of 2% potassium lactate, which resulted only in a very slight reduction of the pH, caused a statistically significant increase of released liquid during heat processing. In these batches radiation treatment

promoted this effect. Fat release was enhanced significantly by the a_w-reducing additives. Radiation treatment tended also to enhance the fat release.

The exudate in the intensely heat-treated ($F_0 = 5.6$) samples was more brownish than that in samples heated by mild treatment ($F_0 = 0.86$). The fat-phase of the ascorbic acid-containing samples showed a reddish colour.

2.2.6. The consistency of canned luncheon meat as affected by the combination treatments. The softness values of samples treated with various combinations are shown in Table 7.

Table 7

Consistency of canned luncheon meat as affected by various treatments
(Number of replicate measurements per treatment: 13)

Code	Treatment	Average softness in dispersitron units (penetration depth)	
1	$F_0 = 5.6$	32.5[a]	a
2	$F_0 = 0.86$	32.4	a
23	3 kGy + $F_0 = 0.86$	32.1	a
3	AsH + $F_0 = 0.86$	28.3	a
33	AsH + 3 kGy + $F_0 = 0.86$	20.2	b
4	1.5% KCl + $F_0 = 0.86$	18.3	b
43	1.5% KCl + 3 kGy + $F_0 = 0.86$	20.7	b
5	2.0% Potassium lactate + $F_0 = 0.86$	16.5	b
53	2.0% Potassium lactate + 3 kGy + $F_0 = 0.86$	16.6	b

LSD = 5.42
[a] Means not followed by the same letter are significantly different from each other ($\alpha \leq 0.05$)
For key of abbreviations, see Table 1.

The samples containing a_w-reducing additives were statistically significantly less soft than the other ones.

2.2.7. The colour of canned luncheon meat as affected by the treatments. The CIELAB colour characters of the canned products three weeks after treatments are summarized in Table 8.

The data in Table 8 demonstrate that irradiated samples tended to give higher red character (a^*-values) as compared to the $F_0 = 5.6$ heat treated control, while the yellow character (b^*-value) of the latter was higher than that of less intensively heated samples. The colour differences between the control and the less heated or combined-treated samples (the range of ΔE^*_{ab}-values was 1.84–3.30) were visible by the naked eye. Samples containing ascorbic acid tended to show higher L*-values than those of the $F_0 = 5.6$ control. No significant difference between various experimental batches was found regarding the C* colour saturation value. The ΔH^*-values between the control and other samples varied between -1.00 (treatment: AsH + 3 kGy + $F_0 = 0.86$) and -2.41 (treatment: KCl + 3 kGy + $F_0 = 0.86$).

Table 8

Colour characteristics of canned luncheon meat samples as affected by various treatments

Code	Treatment	CIELAB colour characters					
		a*	b*	L*	C*	h	ΔE^*ab
1	$F_0 = 5.6$	12.2–13.7 a	11.5–12.9 bc	55.3–55.5 b	16.8–18.8 ab	42.2–44.2 c	—
2	$F_0 = 0.86$	12.5–14.0 ab	9.9–10.8 a	54.4–56.4 ab	16.2–17.5 a	36.0–39.9 ab	2.13
23	3 kGy + $F_0 = 0.86$	14.0–15.3 bc	10.9–12.0 abc	54.3–55.5 ab	17.8–19.3 b	37.3–38.9 ab	1.84
3	AsH + $F_0 = 0.86$	13.7–15.7 abc	11.0–12.7 bc	55.3–59.2 b	17.9–19.9 b	36.4–41.4 ab	3.30
33	AsH + 3 kGy + $F_0 = 0.86$	13.3–14.4 ab	11.0–12.3 bc	56.3–57.5 c	17.4–18.8 ab	38.4–41.6 b	2.73
4	KCl + $F_0 = 0.86$	13.5–15.0 abc	9.6–10.9 a	53.7–55.9 ab	16.7–18.4 ab	34.3–37.1 a	2.36
43	KCl + 3 kGy + $F_0 = 0.86$	14.8–15.9 c	10.6–11.5 ab	52.9–54.6 a	18.6–19.4 b	33.9–37.5 a	2.75
5	Potassium lactate + 3 kGy	13.4–15.0 abc	10.6–11.7 ab	54.5–56.9 ab	17.2–18.9 ab	36.7–39.4 ab	2.11
55	Potassium lactate + 3 kGy + $F_0 = 0.86$	14.1–15.8 bc	11.8–12.6 bc	53.8–55.9 ab	18.6–20.0 b	37.2–41.2 ab	2.04

[a] 95% confidence intervals
Colour characters marked by the same letter in the same column are not significantly different from each other ($\alpha \leq 0.05$)
For key of abbreviations, see Table 1

Table 9

Results of non-parametric sensory testing of canned luncheon meat samples
Number of panelists: 7

Code	Treatment	Average scores in arbitrary units		
		Flavour (smell)	Taste	Consistency
1	$F_0 = 5.6$	2.74 a	2.31 a	2.49 a
2	$F_0 = 0.86$	2.89 a	2.61 a	3.41 a
23	3 kGy + $F_0 = 0.86$	2.56 ab	1.83 ab	2.36 a
3	AsH + $F_0 = 0.86$	0.84 c	1.76 ab	2.70 a
33	AsH + 3 kGy + $F_0 = 0.86$	1.39 dc	1.63 ab	2.86 a
4	1.5% KCl + $F_0 = 0.86$	2.51 ab	1.21 b	3.07 a
43	1.5% KCl + 3 kGy + $F_0 = 0.86$	2.11 abc	0.86 b	2.44 a
5	1.0% Potassium lactate + $F_0 = 0.86$	1.94 abc	1.20 b	3.20 a
53	2.0% Potassium lactate + 3 kGy + $F_0 = 0.86$	1.36 bc	1.41 ab	2.13 a
	$LSD_{0.05}$	1.30	1.24	1.24

Means not followed by the same letter in the same column are significantly different from each other ($\alpha \leq 0.05$)
For key of abbreviations, see Table 1.

2.2.8. Sensory quality. Table 9 summarized the results of the sensory testing of luncheon meat samples immediately after the preservation treatments.

The data in Table 9 show that the panel could not observe a statistically significant difference in sensory qualities between the traditionally canned and the mildly heat-treated samples. No statistically significant differences were noted when 3 kGy-irradiation preceeded heat treatment as compared to similar samples heat-treated only. The pH-reduction by ascorbic acid to pH 5.4–5.7 caused a different flavour as compared to the non-acidified controls. Supplementing the regular salt content of luncheon meat with 1.5% KCl or 2.0% potassium lactate resulted in statistically significant lower taste-scores due to their strong saltiness and bitterish off-taste. The instrumentally detectable differences in the consistency of various samples (see 2.2.6.) were not recognized by the sensory testing panel.

2.2.9. Thiamine content as affected by heat processing or combination of irradiation and mild heat treatment. Estimation of the thiamine content was performed only with samples from three treatments, namely the $F_0 = 5.6$ ones representing traditional canning, samples given a mild heat treatment ($F_0 = 0.86$) and those having been exposed to the combined treatment of 3 kGy plus $F_0 = 0.86$. The results are given in Table 10.

Our preliminary data in Table 10 show that thiamine retention in traditionally canned ($F_0 = 5.6$) luncheon meat was only approx. 6% lower than that in the mildly heated ($F_0 = 0.86$) batch. Thiamine retention in the com-

Table 10

Thiamine content of canned luncheon meat samples from three experimental batches

Code	Treatment	Thiamine content (μg per 100 g)
1	$F_0 = 5.6$	590
2	$F_0 = 0.86$	630
53	3 kGy + $F_0 = 0.86$	430

bined treated (3 kGy + $F_0 = 0.86$) batch was approx. 32% lower compared to that, which received only a mild heat treatment.

In recent US-studies 840–880 μg thiamine per 100 g of raw minced pork were found (FOX et al., 1989; JENKINS et al., 1989) and an exponential loss of thiamine as a function of irradiation dose and subsequent cooking. From these data, an approx. 33% thiamine-loss can be derived for ground pork irradiated at a 3 kGy dose at 20 °C. WILSON (1959) reported a thiamine-loss of 25% in beef irradiated with 3 kGy X-rays at room temperature.

3. Discussion and conclusion

The experimental data show that although a radiation dose of 3–4 kGy had little direct lethal effect on *Clostridium sporogenes* spores in luncheon meat, the same radiation treatment combined with a heat treatment of $F_0 = 0.8$ synergistically controlled this important spoilage organism. This applied particularly, when the pH was reduced to 5.5–5.7. One can speculate that *Clostridium botulinum* spores of similar resistance characteristics will behave similarly.

In our second and third experimental series such combinations resulted in about the same microbiological effect (expressed by the *Pr* value) as that estimated for those shelf-stable canned luncheon meats which have considerably higher salt and nitrite contents (HAUSCHILD & SIMONSEN, 1985). More drastic reduction of the pH seems counter-productive because of the adverse effect of pH-reduction on taste, water holding capacity, and consistency of luncheon meat. Additives used to reduce a_w and increase thereby the inhibition of surviving spores proved to be little promising, due to their adverse sensorial effects. Therefore, further research is necessary to establish microbiologically efficient combinations which affect the thiamine content and sensory quality of the product to a lesser extent.

*

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EFFECT OF COOKING ON NUTRIENTS, MICROBIAL AND SENSORY PROPERTIES OF SKIMMED MILK AND RAYEB KISHK

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The protein content decreased by 2.4 and 3.2% during cooking of skimmed milk and Rayeb Kishk, respectively. Rayeb Kishk was higher in both volatile and non-volatile organic acids than skimmed milk Kishk. The percentages of butyric, propionic, lactic and succinic acids were decreased, but acetic and formic acids disappeared on cooking. All the essential amino acids were at levels adequate for a nutritious food in both types of Kishk. Total and free amino acids either remained unchanged or underwent a slight drop on cooking. The number of aerobic mesophilic bacteria decreased, while lactic acid bacteria and yeasts were destroyed during cooking of Kishk. Utilization of inexpensive skimmed milk in Kishk preparation is a way for raising its protein value and consumer acceptability.

Keywords: skimmed milk, Rayeb Kishk, milk properties, heat effect on milk

Fermented foods are important components of diets in many parts of the world, especially South-East Asia, the Near East and some parts of Africa (VAN VEEN & STEINKRAUSE, 1970). Kishk is one of the most popular fermented milk-wheat mixtures stored in the form of dried balls, brownish in colour and having a rough surface and a hard texture (ATIA & KHATTAB, 1985). It has different names in different areas such as Tarhana in Turkey, Kishk in Egypt, Palestine, Syria, Lebanon and Jordan and Kushuk in Iraq (VAN VEEN & GRAHAM, 1969). In general, two to three parts of yoghurt are mixed with one part of wheat and fermented for a week, and the product is dried in the sun or shade. Other ingredients may be added to the cereal milk combination. For example, turnips are added in Iraq (ALNOURI et al., 1970) as well as tomato paste, red peppers and chopped onions (VAN VEEN & GRAHAM 1969). HAMAD and FIELDS (1982) made a new type of Kishk from whey. An acceptable soy-based Kishk (9.1% moisture, 17.2% protein) was prepared by mixing soy-yoghurt with bulgur at a ratio of 2 : 1 (v/w) (HAFEZ & HAMADA 1984). HASSAN and HUSSEIN (1987) prepared Kishk by replacing milk protein with soy bean protein as well as replacing wheat with chickpeas to improve the nutritional quality of the final product. EL-SADEK and co-workers (1958) correlated the development of carboxylic acids (acidity) during fermentation with Kishk acceptability. SALAMA (1988) investigated the possibility of preparing inexpensive Kishk by using skimmed milk instead of yoghurt. Kishk is a

balanced food with excellent keeping quality, richer in B-vitamins than either milk or wheat and well adapted to hot climates by its content of lactic acid (HAMAD & FIELDS, 1982).

The chemical composition of different dry kishk samples obtained from middle and upper Egypt local markets were studied by EL-SADEK and co-workers (1958), MORCOS and co-workers (1973), and ATIA and KHATTAB (1985). Kishk is consumed uncooked either alone or with bread as a complete meal, fried by itself or with eggs, or included as an ingredient in meat and chicken soups (MORCOS et al., 1973) as well as in the form of cooked Kishk dishes (HASSAN & HUSSEIN, 1987).

This work was carried out to evaluate the effect of cooking on the nutrient content, free amino acids, organic acids and microbial counts of skimmed milk Kishk, of which little is known.

1. Materials and methods

1.1. Materials

1.1.1. Polished wheat grain. Polished wheat grains were bought on the local market in Alexandria.

1.1.2. Cow liquid skimmed milk. Cow liquid skimmed milk was obtained from the Dairy Department, Faculty of Agriculture, University of Alexandria.

1.1.3. Rayeb milk. Rayeb milk was bought on the local market in Alexandria. Fresh milk is put in an earthenware left undisturbed in a warm dark place until the cream rises and the milk coagulates. The cream layer is removed and the curd is consumed fresh as Rayeb milk.

1.1.4. Other ingredients. Salt, onion, olive oil and poultry broth were added as indicated under "Organoleptic evaluation".

1.2. Methods

1.2.1. Preparation of kishk. Preparation steps of skimmed milk Kishk are listed in Fig. 1. Rayeb Kishk was prepared in the same way, except that Rayeb milk was used instead of fermented skimmed milk. Part of the dry Kishk was ground in a wily mill to pass through 45 mesh sieve and kept in a sterilized Kilner jar at 4 °C until used. The remaining part was soaked in water (1 : 3, w/v) for one hour, cooked for 10 min, dried, milled and sieved for further analysis.

1.2.2. Chemical examination. Moisture, ether extract and ash contents were determined according to the standard methods A. O. A. C. (1980). Crude protein, non-protein nitrogen and crude fiber were determined as described by PEARSON (1976). Calcium, magnesium, manganese, copper and iron were

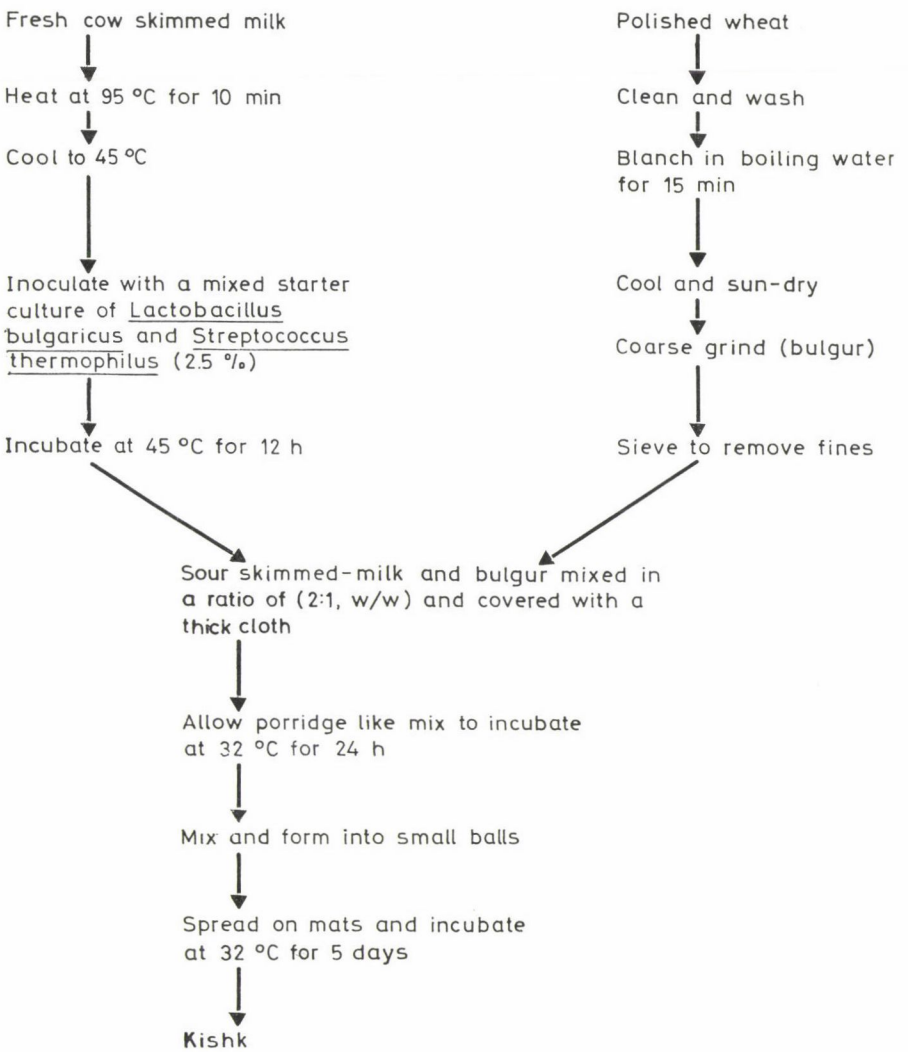

Fig. 1. Flow sheet for the preparation of skimmed milk Kishk

determined by using a Perkin Elmer atomic absorption spectrophotometer (Model 2380), according to A. O. A. C. (1980). Phosphorus was estimated colorimetrically as described by A. A. C. C. (1970). The pH value was measured using pH meter (Orion research ion analyser/Model 407A, USA). Titratable acidity was determined according to the method of EGAN and co-workers (1981), and volatile was estimated using a modified version of the method used by ZEIN (1963). Total amino acids were determined after acid hydrolysis according to the method of MOORE (1958) using a Beckman amino acids

analyser (Model 119 CL). Free amino acids were determined by the sulfosalicylic acid method of HAMILTON (1962).

Organic acids were extracted and determined as follows: Ten g of the ground Kishk sample were extracted with 0.2 *N* sulphuric acid, centrifuged at 3000 r.p.m. for 5 min and filtered. Free acids in an aliquot of the extract were transformed to their sodium salts, then the solution was evaporated to dryness on a steam bath. The modified silica gel column method described by RAGAEE (1984) was used for separation and determination of organic acids. The eluting system used was water equilibrated n-butanol in benzene in which the polarity was raised progressively by increasing the percentage of butanol. This was executed successively in the following manner: three consecutive volumes, 20 cm^3 each, then 5 consecutive volumes, 30 cm^3 each of the eluent was applied. The gradations in butanol concentration were 1, 2, 5, 10, 14, 16 and 50% with respect to benzene in the eight eluents, respectively. The eluting solvent was passed through the column so as to allow for the passage of effluent at the rate of 2–3 cm^3 min^{-1}. The collected fractions, 4 cm^3 each, were titrated with 0.005 *N* sodium hydroxide. A standard curve was constructed using a mixture of known amounts of butyric, propionic, lactic, acetic, formic and succinic acids whose positions were previously determined and the recovery of each acid was calculated (SALAMA, 1988).

1.2.3. Microbiological examinations. Several dilutions of ground Kishk were prepared and microorganisms examined by the pour plate technique. Standard plate count agar, acidified, potato dextrose agar, and lactic agar were used for counting total bacteria, yeasts and lactic acid bacteria, respectively (SPECKS, 1976).

1.2.4. Organoleptic evaluation. Kishk samples were separately cooked using the customary Egyptian method. Dry Kishk balls were soaked in tap water (1 : 3, w/v) for 1 h, then cooked with poultry broth for 10 min, fried chopped onion, salt and olive oil were added to the Kishk after cooking. Organoleptic tests were conducted according to GRISWOLD (1962). Results were statistically analysed using the non-parametric method of HOLANDER and WOLFE (1973) for comparison between the treatments.

2. Results and discussion

2.1. Chemical analysis

Data in Table 1 indicate that skimmed milk Kishk was higher in protein and non-protein nitrogen and lower in ether extract, crude fiber and ash contents than Rayeb Kishk. The wide variation in the ether extract is related to the original fat content of milk used in Kishk preparation. Phosphorus and calcium

Table 1

Chemical composition of dry and cooked Kishks
(on dry basis)

Constituents (%)	Skimmed milk Kishk		Rayeb Kishk	
	Dry	Cooked	Dry	Cooked
Moisture	9.35	82.20	9.66	81.90
Crude protein	17.90	17.47	16.80	16.26
Non-protein nitrogen	0.40	0.34	0.30	0.27
Ether extract	1.83	1.80	4.61	4.56
Crude fiber	2.35	2.38	2.69	2.73
Ash	8.70	8.81	9.90	9.98
Minerals:				
P (mg per 100 g)	375.02	376.01	359.09	360.12
Ca (mg per 100 g)	108.72	109.54	131.39	132.02
Fe (mg per 100 g)	3.06	2.97	5.14	5.10
Mn (mg per 100 g)	2.32	2.20	2.70	2.69
Cu (mg per 100 g)	0.59	0.62	0.70	0.74
Mg (mg per 100 g)	53.83	54.33	57.50	57.68
Nitrogen free extract[a]	69.22	69.20	64.40	66.20

[a] Calculated by difference

constituted the bulk of the six mineral analysed, while copper represented the lowest element content in the two Kishk types. The change in protein content was small, amounting to 2.4 and 3.2% after cooking of skimmed milk and Rayeb Kishk, respectively. The pH values in the two Kishk types were very close to those reported in the literature for Kishk preparation by sun drying, pH 3.8–4.2 (EL-SADEK et al. 1958), HAMAD and FIELDS (1982) and HAFEZ and HAMADA (1984), except for the slightly higher value of 4.5 for skimmed milk Kishk (Table 2). The moisture content of the final products varied within a narrow range: 9.35–9.66. These values were comparable to moisture levels in Kishk made by sun drying. HAMAD and FIELDS (1982) prepared a yoghurt Kishk and whey based Kishk with 9.4 and 9.7% moisture, respectively. The relatively low moisture content of the Kishk accounts for the moderate keeping quality of these products. However, it was not low enough to prevent deterioration if the product was stored for long periods. Low moisture content and low pH, together, are the prime factors in increased shelf life of these products and are considered to be a safeguard against the growth of any pathogenic micro-organisms (WANG & HESSELTINE, 1981). Cooked Rayeb Kishk was more acidic than cooked skimmed milk Kishk, including both titratable and volatile acidities (Table 2).

Table 2

Acidities and organic acids contents of dry and cooked Kishks
(on dry basis)

Kishk sample	pH		Titratable acidity (% as lactic acid)		Volatile acidity (% as acetic acid)	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Dry skimmed milk Kishk	4.5	0.10	1.63	0.08	0.240	0.010
Cooked skimmed milk Kishk	5.2	0.20	1.08	0.03	0.005	0.001
Dry Rayeb Kishk	3.9	0.10	2.02	0.03	0.300	0.020
Cooked Rayeb Kishk	4.8	0.05	1.41	0.05	0.009	0.002

Kishk sample	Organic acid (g per 100 g dry basis)					
	Butyric	Propionic	Acetic	Formic	Lactic	Succinic
Dry skimmed milk Kishk	0.120	0.198	0.149	0.078	0.930	0.100
Cooked skimmed milk Kishk	0.096	0.172	—	—	0.720	0.062
Dry Rayeb Kishk	0.160	0.418	0.186	0.090	1.008	0.124
Cooked Rayeb Kishk	0.120	0.344	—	—	0.860	0.084

The results are average of three replicates

2.2. Organic acids

The characteristic acid flavor of Kishk survives in mixed foods and is particularly appreciated in hot weather. Moreover, the organic acids produced during fermentation, not only influence the flavor of the product, but also deeply affect the microbial action and the state of the proteins and consequently the texture of final product (EL-SADEK et al. 1958). There is no information in the literature with respect to the organic acids in Kishk. Table 2 shows, that Rayeb Kishk in general was higher in both volatile and non-volatile organic acids than skimmed milk Kishk. It can be seen that lactic acid dominates in skimmed milk Kishk, while lactic and propionic acids prevail in Rayeb Kishk, with formic acid being very low in both products. Cooking of Kishk results in diminution the contents of butyric; propionic, lactic and succenic acids with the disappearance of acetic and formic acids. However, the drop did not follow a unified pattern in both types of Kishk. Several factors must play a role such as that of volatilization, transformation and/or decomposition (GEERVANI & THEOPHILUS, 1980).

2.3. Total and free amino acids

The amino acids content of the two Kishk types are presented in Table 3. All the essential amino acids were at reasonable level with those specified in the FAO/WHO/UNU pattern of young children (FAO/WHO/UNU, 1985). Glutamic acid, proline and aspartic acid were at the highest concentration in the two Kishk types. The total amino acids either remained unchanged or underwent a slight drop on cooking.

Table 3

Total amino acids of dry and cooked Kishks
(g per 16 g nitrogen)

Amino acid	Skimmed milk Kishk		Rayeb Kishk		FAO/WHO/UNU pattern
	Dry	Cooked	Dry	Cooked	
Essential					
Lysine	5.42	5.30	5.94	5.66	5.50
Methionine	2.65	2.60	3.48	3.42	3.42
Cystine	2.94	2.80	2.74	2.71	
Threonine	4.04	4.01	3.31	3.28	3.40
Leucine	8.46	8.22	6.48	6.39	6.60
Phenylalanine	2.39	2.27	1.65	1.60	6.00
Tyrosine	3.78	3.70	4.39	4.35	
Isoleucine	2.86	2.77	2.84	2.80	2.80
Valine	3.49	3.45	3.51	3.50	3.50
Non-essential					
Histidine	2.75	2.73	3.33	3.30	
Arginine	1.84	1.80	1.41	1.40	
Aspartic acid	6.97	6.81	6.77	6.60	
Glutamic acid	28.09	28.0	30.08	30.04	
Serine	4.98	4.86	5.94	5.42	
Proline	13.55	13.14	13.71	13.68	
Alanine	2.45	2.37	1.12	1.10	
Glycine	3.45	3.39	3.73	3.54	

Rayeb Kishk had a high content of the free amino acids, threonine, glutamic acid, proline, glycine, alanine, leucine, isoleucine and tyrosine whose level was 1.05–2.0 folds that in skimmed milk Kishk, which was rich in free arginine, histidine and aspartic acid (Table 4). The other remaining free amino acids were similar in both. The cooking of Kishk leads to decrease the level of the free aspartic acid, serine, glutamic acid, leucine, isoleucine, lysine, histidine and arginine. This can be attributed to the reaction of the free amino acids with sugars (DONOSO et al., 1962) as well as to the destruction during cooking (GEERVANI & THEOPHILUS, 1980) as is the case with valine and phenylalanine.

Table 4

Free amino acids content of dry and cooked Kishks
(g per 16 g nitrogen)

Amino acid	Skimmed milk Kishk		Rayeb Kishk	
	Dry	Cooked	Dry	Cooked
Aspartic acid	0.193	0.180	0.183	0.167
Threonine	0.029	0.028	0.039	0.030
Serine	0.091	0.080	0.095	0.081
Glutamic acid	0.224	0.209	0.242	0.204
Proline	0.122	0.120	0.140	0.130
Glycine	0.235	0.233	0.246	0.231
Alanine	0.058	0.057	0.069	0.060
Cystine	—	—	—	—
Valine	0.051	—	0.056	—
Methionine	—	—	—	—
Isoleucine	0.010	0.006	0.020	0.011
Leucine	0.078	0.067	0.084	0.070
Tyrosine	0.036	0.032	0.048	0.044
Phenylalanine	0.013	0.007	0.012	—
Histidine	0.015	0.011	0.010	0.004
Lysine	0.029	0.021	0.028	0.016
Arginine	0.020	0.005	0.011	0.006

2.4. Microbiological examination

The results in Table 5 show that skimmed milk Kishk contained lower count of lactic acid bacteria than Rayeb Kishk, but both were almost equal in total mesophilic bacteria and yeasts. This may be due to the fact that skimmed milk Kishk is made of milk fermented using a pure culture, while Rayeb Kishk utilizes widely fermented milk. Cooked skimmed milk Kishk was slightly less in total mesophilic bacterial count than cooked Rayeb Kishk. Lactic acid bacteria and yeasts were absent in both types of Kishk after cooking due to destruction by heat.

Table 5

Microbial counts in dry and cooked Kishks
(CFU[a] cm^{-3})

Kishk sample	Total aerobic mesophilic bacteria	Lactic acid bacteria	Yeasts
Dry skimmed milk Kishk	2×10^3	6.4×10^6	1.01×10^3
Cooked skimmed milk Kishk	1×10^2	—	—
Dry Rayeb Kishk	1.8×10^3	2.2×10^7	8×10^2
Cooked Rayeb Kishk	1.5×10^2	—	—

[a] Colony Forming Unit

2.5. Organoleptic evaluation

The results of the sensory evaluation of cooked skimmed milk and Rayeb Kishk are presented in Table 6. Analysis of variance revealed no significant differences for colour and consistency between both types of Kishk. The mean score of skimmed milk-Kishk flavour was significantly ($P < 0.05$) higher than

Table 6

Mean sensory scores of cooked skimmed milk and Rayeb Kishks

Kishk sample	Colour		Consistency		Flavour	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Skimmed milk Kishk	7.43	0.22 a	7.00	0.10 a	7.29	0.36 a
Rayeb Kishk	7.21	0.15 a	6.60	0.30 a	6.10	0.20 b

Each value is the mean and standard deviation. Means in a column followed by the same letter are not significantly different ($P \leq 0.05$)

that of Rayeb Kishk. Many of panelists commented on the more strongly acid taste of cooked Rayeb Kishk, which was possibly the cause of the decrease in its acceptability.

3. Conclusion

The practical aspect of this study shows how the incorporation of inexpensive skimmed milk in fermented cereal products as Kishk is a way of raising their protein value and consumer acceptability. Furthermore, it extends the availability of milk which fluctuates from season to season. Thus such products can be introduced in programmes of school feeding in rural areas. Its value would gradually become better known and hence a large scale programme could be launched to exploit fully the potentialities of milk to the benefit of all sections of the community.

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CHANGE OF FOAM PROPERTIES OF GELATIN SOLUTIONS UNDER DIFFERENT CONDITIONS

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Certain organoleptic properties of some foods can be favourably influenced by using of some foaming agent. A lot of food additives were synthetized for this purpose.

The aim of our investigations was to clear up the best conditions (heat treatment, pH value) under which the foaming properties of gelatin solutions could be advantageously improved. Heat treatment was carried out at different temperatures (50, 70 °C). The pH values varied between 4.6 and 12.0. Heat treatment time applied were: 30, 60, 90 and 120 min.

The best foam properties (foaming capacity, foam stability and foam quality) were shown by the samples containing 2.5% gelatin and adjusted to pH value 6.0 after heat treatment at 50 °C. The conditions of the heat treatment influenced characteristically the foam properties.

Keywords: foam consistency, foaming capacity, foam stability, foam structure, gelatin

The foaming properties of proteins are influenced by many factors such as concentration, surface, structure, pH, temperature and certain substances (CHERRY, 1981).

Beside the traditional egg white, the gelatin as an other natural product is used extensively for whipping. Other protein products were also made for this purpose (AKERS, 1976). The foaming properties of these different samples show quite different characters. They can be influenced by the varying of some factors of the treatment process: pH, conditions of heat treatment, using of special other substances, etc. (LAWHTON & CATER 1971; TYBOR et al., 1975).

Experiments were carried out in our laboratory to produce a gelatin product with improved foaming properties. We investigated the effect of heat treatment and pH as influencing factors.

1. Materials and methods

1.1. Material

50 cm^3 of gelatin dispersions (1.5 or 2.5%) dispersed in dist. water, allowed to stand overnight at 20 °C.

1.2. Methods

1.2.1. Heat treatment and pH. Temperatures: 50 °C and 70 °C ±0.2 °C (control 20 °C); time: 30, 60, 90 and 120 min; pH values: 4.6, 6.0, 7.0, 8.0, 9.0, 10.0 and 12.0 5% NaOH and 5% HCl solutions were used for adjustment carried out previous to heat treatment.

1.2.2. Foaming conditions. Time: 4 min; temperature: 20 °C; device: GIRMI electric blender (foaming at maximum rotation rate).

1.2.3. Methods used to test the foam properties. Foaming capacity (FC): foam volume (cm^3) measured at the end of foam production; foam stability (FS): the change of foam volume during storage at 20 °C ($cm^3\ min^{-1}$). Foam quality: foam consistency (solid, viscous, fluid) and foam structure (the size of the bubbles — fine (diameter under 0.5 mm); medium (between 1–3 mm) and coarse (diameter above 3 mm); fluid leakage (FL)% $= \frac{F_t}{F_0} \times 100$. ($F_0$: initial gelatin solution, cm^3; F_t: dropped fluid volume during storage, cm^3).

1.2.4. Evaluations were carried out by the methods and at the accuracy used in this field of research (HANSEN & BLACK, 1972; BALDWIN & SHINTAVALAI, 1974; ETHERIDGE et al., 1981; HAYAKAWA et al., 1982; TYBOR et al., 1975; KITABATAKE & DOI, 1988; BERA & MUKHERJEE, 1989).

Accuracy of the reading is 5 cm^3 therefore the standard deviations are relatively high. Values given are the means of 3 measurements (n = 3).

2. Results

1.2. Foaming capacity

The effect of different conditions of heat treatment on the FC in the case of 1.5% gelatin solution is presented in Table 1.

The data show a characteristic effect of the pH values and the heating times on the FC values. The best results were obtained with the solutions heat treated at 70 °C for 60 min. The pH values of these solutions had been adjusted to pH 4.6, 6.0 and 8.0.

2.2. Foam stability

Data of the measurements are shown in Figs. 1–5.

The most stable foam systems were achieved in the solutions which were heat treated at 50 °C and 70 °C for 30 min.

2.3. Foam quality

The foam quality of the above solutions was characterized by the consistency and structure of foams. The observations are collected in Table 2.

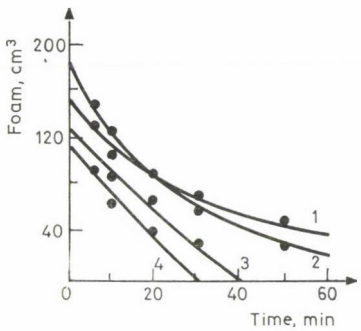

Fig. 1. FS values of 1.5 w% gelatin solution without heat treatment (20 °C, 0 min) at different pH values. 1: pH = 7.0; 2: pH = 4.6; 3: pH = 8.0; 4: pH = 9.0

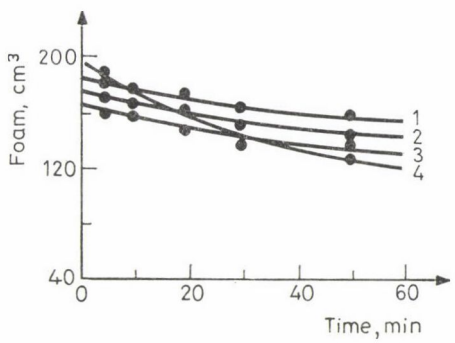

Fig. 2. FS values of 1.5 w% gelatin solutions heat treated at 50 °C for 30 min at different pH values. 1: pH = 9.0; 2: pH = 8.0; 3: pH = 7.0; 4: pH = 4.6

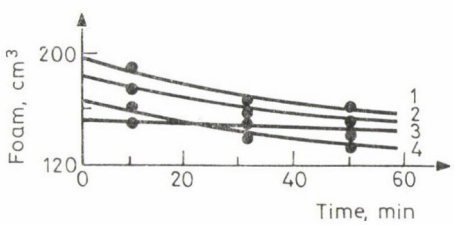

Fig. 3. FS values of 1.5 w% gelatin solutions heat treated at 70 °C for 30 min at different pH values. 1: pH = 9.0; 2: pH = 8.0; 3: pH = 4.6; 4: pH = 7.0

The samples of highest quality were those heat treated at 50 °C for 30 min, independent of the pH value. Similar properties were observed in the solution which was treated at 70 °C for 60 min. The pH value of this sample was adjusted to 9.0 previous to heat treatment.

Table 1

The change of FC (cm^3) of 1.5 wt% gelatin solutions under different conditions

	Time of heat treatment (min)													
	0		30				60				90			
	Temperature of heat treatment (°C)													
	20		50		70		50		70		50		70	
pH	FC	SD	FC	SD	FC	SD	FC	SD	FC	SD	FC	SD	FC	SD
4.6	175	5.00	—	—	—	—	—	—	200	13.23	160	5.00	210	5. 00
7.0	165	8.66	150	5.00	155	13.23	150	5.00	200	13.23	—	—	—	—
8.0	145	5.00	160	8.66	170	10.00	160	13.23	200	13.23	165	10.00	180	5.00
9.0	140	10.00	160	8.66	170	10.00	160	13.23	180	13.23	163	10.00	180	5.00
10.0	—	—	—	—	—	—	160	13.23	150	10.00	165	13.23	180	8.66
12.0	—	—	—	—	—	—	160	10.00	10	13.23	170	10.00	210	5.00

Note: The foaming process was carried out in a glass vessel of 400 cm^3. The accuracy of the reading was 5 cm^3.
SD: standard deviation

Table 2

The change of foam quality of 1.5 wt% gelatin solution

Conditions of treatment			Foam quality	
Time (min)	Temperature (°C)	pH	Consistency	Structure
0	20	4.6	viscous	medium
0	20	6.0	fluid	coarse
0	20	8.0	fluid	coarse
0	20	9.0	fluid	coarse
30	50	4.6	solid	fine
30	50	7.0	solid	fine
30	50	8.0	solid	fine
30	50	9.0	solid	fine
60	70	4.6	viscous	medium
60	70	8.0	viscous	medium
60	70	9.0	solid	fine
60	70	10.0	fluid	coarse
60	70	12.0	fluid	coarse
90	70	4.6	viscous	fine
90	70	8.0	viscous	fine
90	70	9.0	fluid	medium
90	70	10.0	fluid	coarse
90	70	12.0	no foam	no foam

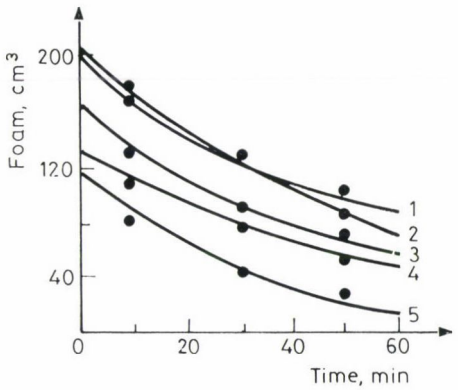

Fig. 4. FS values of 1.5 w% gelatin solutions heat treated at 70 °C for 60 min at different pH values. 1: pH = 4.6; 2: pH = 8.0; 3: pH = 9.0; 4: pH = 10.0; 5: pH = 12.0

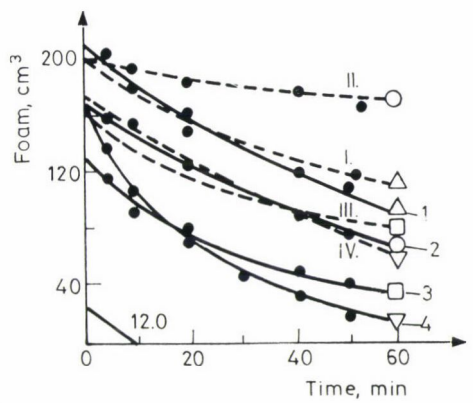

Fig. 5. FS values of 1.5 w% gelatin solutions heat treated at 70 °C for 90 min at different pH values (full lines: 1: pH = 4.6; 2: pH = 8.0; 3: pH = 9.0; 4: pH = 10). Changes in FS values of the solutions in the case of pH adjustments after heat treatment to pH = 6.0 (dotted lines: I. pH = 4.6 to 6.0; II. pH 8.0 to 6.0; III. pH 9.0 to 6.0; IV. pH = 10.0 to 6.0)

2.4. Effect of pH adjustmeni on the foam quality after heat treatment

Investigations were carried out to observe the change of foam properties of 1.5 w% gelatin solutions which were heat treated at a predetermined pH value and after cooling adjusted to pH 6.0. The heat treatment was carried out at 70 °C for 90 min.

Data and observations can be seen in Table 3. The change in FS values is shown in Fig. 5 (dotted lines).

Those solutions which had been adjusted to pH values of 4.6, 8.0 and 9.0 showed characteristic improvement in their foam stability and quality related to the controls (samples without pH change after heat treatment).

Table 3

The effect of the pH on the foaming properties in 1.5 wt% heated solutions

(Heat treatment at 70 °C for 90 minutes)

pH value		FC (cm^3)	Foam quality	
before heat treatment	after heat treatment		Consistency	Structure
4.6	4.6	210	viscous	fine
4.6	6.0	180	solid	fine
8.0	8.0	180	viscous	fine
8.0	6.0	200	solid	fine
9.0	9.0	160	fluid	medium
9.0	6.0	150	viscous	fine
10.0	10.0	180	fluid	coarse
10.0	6.0	no foam	—	—
12.0	12.0	25	—	—
12.0	6.0	70	fluid	coarse

2.5. Investigations with 2.5 wt% gelatin solutions

Gelatin solutions (pH = 4.6) were investigated after heat treatment at 50 °C and 70 °C for 30, 60, 90 and 120 min. The experiments were repeated at a pH value of 6.0 which was adjusted after heat treatment.

The FS and FL values are shown in Figs. 6 and 7.

The evaluations of foam structures are collected in Tables 4 and 5.

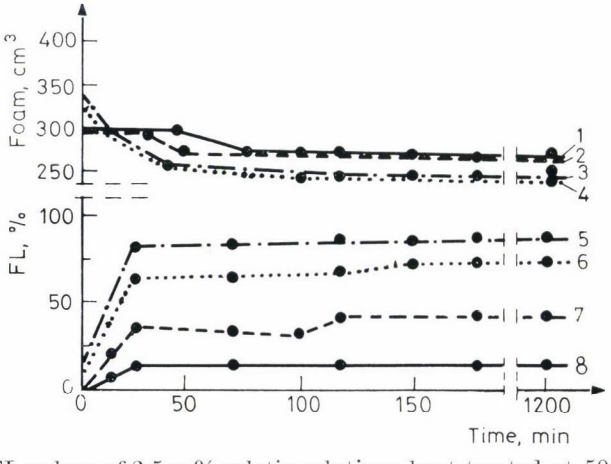

Fig. 6. FS and FL values of 2.5 w% gelatin solutions heat treated at 50 °C for different times. 1: 30, 2: 60 min; 3: 90, 4: 120 min: 5: 120 min; 6: 90 min; 7: 60 min; 8: 30 min

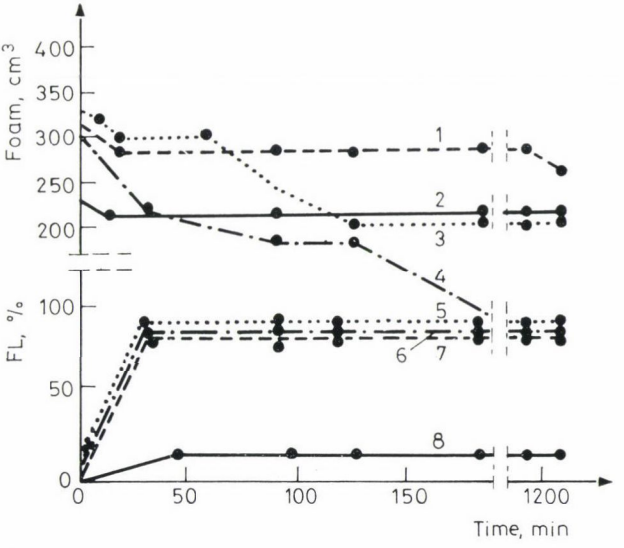

Fig. 7. FS and FL values of 2.5 w% gelatin solutions heat treated at 70 °C for differential times. 1: 60 min; 2: 30 min; 3: 90 min; 4: 120 min; 5: 90 min; 6: 120 min; 7: 60 min 8: 30 min

Table 4

Foam structure of 2.5 wt% gelatin solutions heat treated at 50 °C

Storage time (min)	Heat treatment (min)			
	30	60	90	120
0	fine	fine	very fine	very fine
15	fine	fine	medium	medium
25	fine	fine	medium	coarse
60	fine	fine	coarse	coarse
100	fine	fine	coarse	coarse
150	fine	medium	coarse	coarse
1200	medium	medium	coarse	coarse

Table 5

Foam structure of 2.5 wt% gelatin solutions heat treated at 70 °C

Storage time (min)	Heat treatment (min)			
	30	60	90	120
0	fine	fine	fine	fine
5	fine	fine	medium	coarse
15	fine	fine	coarse	coarse
45	fine	medium	coarse	coarse
100	fine	coarse	coarse	coarse
180	fine	coarse	coarse	coarse
1200	medium	coarse	coarse	coarse

The best FL values were obtained in the samples heat treated only for 30 min.

The foam stability was favourable in the case of a heat treatment at 50 °C. It was practically independent of the time of heat treatment.

The quality of the foam structure and its stability seemed to be the best after a heat treatment of 30 min. No influence of the heat treatment temperature was observed.

Foams prepared from the gelatin solutions which were heat treated under different conditions are shown in Figs. 8 and 9.

Heat treatment at 50 °C (Fig. 8) gave generally more favourable foam properties, than at 70 °C (Fig. 9): the foaming capacity was higher and the foam stability was better.

The change of pH after heat treatment had a favourable effect on the FL values. The samples which were heat treated at 50 °C had no FL value at all independently of the heat treatment time.

The foam stability of each sample was excellent.

A characteristically lower FC value was measured in these samples than in those foamed at the pH value of the heat treatment.

3. Conclusions

It can be established from the results of the experiments with 1.5% gelatin solutions that the foaming properties of gelatin can be characteristically influenced by heat treatment at different pH values.

The highest FC values were obtained with samples which were adjusted to pH values between 4.6 and 8.0 and treated at 70 °C for 90 min.

The samples heat treated at 50 ° and 70 °C for 30 min showed the best FS values independently of the pH.

The highest foam quality was found after heat treatment at 50 °C for 30 min. The pH values of these solutions were varied from 4.6 to 9.0.

Comparing these data to the results obtained with 2.5% gelatin solutions, it can be established that the foam properties depend first of all on the concentration of the gelatin solutions. The gelatin solutions of different concentrations showed quite different foam properties even if treated under the same conditions. It can be concluded from the data, that the 2.5% gelatin solutions give better foam properties. (In earlier experiments we observed jellification in the solutions over 2.5% gelatin content after heat treatment, which made difficult the foaming process (GÁBOR 1990).

Summarizing the results and taking into consideration the different significance of the examined foam properties we established an improvement of the foam properties of the 2.5% gelatin solutions which were adjusted to a pH value of 6.0 after heat treatment at 50 °C.

Fig. 8. FS values and structure of foams obtained in 2 5 w% gelatin solution (pH = 4.6). Heat treatment: 50 °C; time: min: $A_1 = 30$, $A_2 = 60$, $A_3 = 90$, $A_4 = 120$

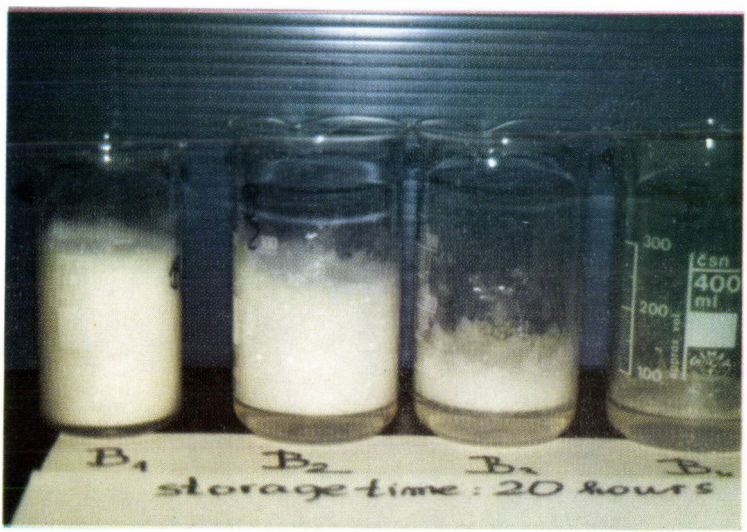

Fig. 9. FS values and structure of foams obtained in 2.5 w% gelatin solution (pH = 4.6). Heat treatment: 70 °C; time, min: $B_1 = 30$, $B_2 = 60$, $B_3 = 90$, $B_4 = 120$

Further examinations are planned to find the best gelatin concentration and discover the connections between the change of the foam properties and the distribution of the molecular weight of the different fractions originated under different conditions.

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ACTIVITIES OF PROTEOLYTIC ENZYMES AT DIFFERENT RIPENING AGES IN ASIAGO PRESSATO CHEESE PRODUCED BY SEVERAL MILK COAGULANTS

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Protein degradation during cheese ripening is catalyzed by the enzymes present in the milk, commercial rennet preparations and those deriving from starter microorganisms. The coagulants used for cheese making show different proteolytic activities in relation to their microbial or animal origin. Asiago pressato cheese was manufactured at a commercial dairy plant according to conventional Italian procedures using various microbial, bovine-microbial and bovine rennets. Acid and neutral protease, acid phosphatase and aminopeptidase activities along with electrophoretic analysis of pH 4.6 insoluble nitrogen compounds were carried out after 10, 20 and 30 days of cheese ripening. Electrophoretic patterns of the cheese samples showed after 10 days of ageing hydrolysis in the β- and α_{s1}- casein fractions with the production of peptides having mobilities similar to those of β–I and α_{s1}–I, respectively. The protein breakdown was gradual from curd to 30-day-old samples. At the same time, acid and neutral protease and aminopeptidase activities, analyzed by two ways (coagulant and ripening time) factorial analysis of variance and Neuman-Keuls test, increased while acid phosphatase activity slightly decreased.

Keywords: Asiago pressato cheese, cheese ripening, coagulant, proteolytic enzymes

Protein degradation during cheese ripening is catalyzed by enzymes naturally present in milk and which derive from either rennet preparations or starter microorganisms. These enzymes display a synergistic proteolytic activity on caseins. Different types of cheese have variable portions of rennet activity remaining in the curd (Holmes et al., 1977; Lawrence et al., 1984; Kamaly & Marth, 1989), which thus affect the cheese ripenig process. For some pressed cheese, like St. Paulin, the role of rennet in ripening is likely to be more important than that of lactic bacteria (Desmazeaud & Gripon, 1977). Green and Foster (1974) showed that the proteolytic activity of rennet in aseptically manufactured Cheddar cheese was present for at least 7 months. Gripon and co-workers (1975) observed that in aseptic curds, rennet, in addition to the cleavage of the Phe_{105}-Met_{106} bond of k-casein and subsequent induction of clotting, also caused the early cleavage of the Phe_{23}-Phe_{24} bond of α_{s1}-casein leading to formation of soluble nitrogen, which regularly increased until the 48th day of ripening. It has been reported (Desmazeaud & Gripon, 1977) that β-casein is hydrolysed during the general process of cheese ripening

and that the β-I component is formed from the cleavage of Ala_{189}-Phe_{190} and Leu_{191}-Tyr_{192} bonds. There is a good deal of evidence that in aseptic curds the role of rennet in amino acid formation is a minor one (DESMAZEAUD & GRIPON, 1977), but it may provide high molecular weight peptides whose subsequent degradation by starters could result in low molecular weight compounds (LAWRENCE et al., 1972; O'KEEFFE et al., 1978). Although several papers deal with the role of different coagulants in the ripening process, studies on casein hydrolysis in model solution are often carried out under conditions which are suboptimal, at least when compared to those existing in cheese (GREEN, 1977). Important parameters which are difficult to control, such as pH, protein concentration, water and salt content and temperature may influence the activity of chymosin and plasmin and consequently the rate of hydrolysis of different caseins and the nature of the products formed (LAWRENCE et al., 1984; GRAPPIN et al., 1985; LAWRENCE et al., 1987) with consequent effect on cheese structure and flavours. However, the role of plasmin in cheese ripening seems to be significant particularly in high-cooked cheeses (GRUFFERTY & FOX, 1988). In a survey on suitable rennet substitutes in Italian cheese manufacture, CORRADINI (1977) observed that all the enzymes from animal rennet were inactivated in Grana and Provolone cheese making. This observation originated mainly from the comparison of the rate of proteolysis in Grana Padano and Crescenza cheeses made either with calf rennet or with *Mucor miehei* coagulant.

The use of liquid, powdered and paste coagulants from different sources (animal, microbial and animal-microbial blend) did not show significant differences in the characteristics of Cheddar cheese (WONG et al., 1977), Provolone cheese (CARINI et al., 1977), Gruyere-type cheese (ANIFANTAKIS et al., 1981), Edam cheese, Emmenthal-, Bel Paese-type cheeses (HUSEK & DEDEK, 1981) and Feta cheese (ALICHANIDIS et al., 1984). In "Pategras" cheese making it was possible to substitute powdered calf rennet by adult bovine rennet or by a microbial coagulant from *Mucor pusillus*, while when using swine pepsin, poor quality cheeses were produced (ZALAZAR et al., 1984).

In this study, an attempt was made to obtain useful information for the production of an Asiago pressato cheese with constant quality characteristics. To assess the coagulant function during cheese ripening, nine different commercial (liquid and dry) coagulants were comparatively studied. Here we report acid and neutral protease, aminopeptidase and acid phosphatase activities, in addition to the electrophoretic patterns of pH 4.6 insoluble nitrogen compounds, determined in Asiago pressato samples during 10, 20 and 30 days of ageing.

1. Materials and methods

1.1. Coagulants

Nine commercial milk clotting preparations, either liquid or powdered, from six different firms were used: one was from *Mucor miehei*, two were from calf rennet–*Mucor miehei* blend (50 : 50) and six were from calf rennets.

1.2. Cheese making procedure

Asiago pressato cheese was manufactured at a commercial dairy plant according to conventional Italian cheese making procedures.

Bulk raw milk was heated at 60 °C for 30 s prior to cheese making. After heat treatment, milk was cooled to 37 °C and 1% mixed starter culture *(Streptococcus lactis, Streptococcus cremoris* and *Lactobacillus bulgaricus)* was added followed by sufficient coagulant so that the curd could be cut after 20–25 min. Following coagulation, the curd was cut and reduced to "apple" size particles scalding at 42 °C and then reduced to "hazelnut" size particles (1.5 cm) scalding at 46 °C for a total cooking time of 6–8 min. The curd was removed from the whey when the latter reached 2.90° S. H. in 50 cm^3, pressed at 3.5–4 kg cm^{-2} for 6–7 h and then treated with dry fine salt for four days. The average salt content was approximatively 2%. The cheese was ripened at 15–16 °C and 80% R. H.

Four cheeses of about 13 kg weight for each coagulant used were obtained from four vats of milk.

1.3. Analytical methods

Cheese samples (20 g) were homogenized with an Ultra-Turrax apparatus in 50 cm^3 ice-cold 0.01 mol l^{-1} citrate buffer pH 6.0. The slurry was centrifuged at 4 °C and 12,000 g for 10 min and then filtered through Whatman No. 4 filter paper. The filtrate was used for enzyme assays. The determinations of enzyme activities were carried out on at least one cheese sample from each separate cheese making trial for each coagulant used (i.e. $n = 4$ for acid and neutral protease; $n = 5$ for acid phosphatase; $n = 7$ for aminopeptidase).

Enzymatic assays were performed as previously described (Spettoli & Zamorani, 1985). Acid and neutral protease activities respectively at pH 4.0 (hemoglobin substrate) and pH 7.5 (casein substrate). Aminopeptidase activity at pH 7.5 on leucine-p-nitroanilide and acid phosphatase activity at pH 5.0, using p-nitrophenylphosphate as substrate.

pH measurements were made with a spear type electrode (Ingold AG Industrie Nord, Urdorf, Switzerland).

Polyacrylamide gel electrophoresis of the pH 4.6 insoluble nitrogen fraction, which largely represents isoelectric precipitated casein and its degradation products (GRIPON et al., 1975) was performed as reported by ZAMORANI and co-workers (1986). Two mg of freeze-dried precipitated nitrogen compounds were solubilized in 1 cm^3 of 25 mmol Tris — 192 mmol glycine containing 30% (w/v) sucrose and electrophoresed in accordance with a partially modified technique of LAEMMLI (1970). The modification concerned the omission of the detergent sodium dodecyl sulphate and the addition of 6 mol urea in both the stacking and separating gels. Acrylamide and bisacrylamide concentrations in the separating gel were 15% and 0.38%, respectively. Fifteen μg of protein was loaded in each slot.

2. Results

The levels of enzyme activities and pH, analyzed by two ways factorial analysis of variance and Neuman-Keuls test, in samples of Asiago pressato cheese, produced with nine different commercial coagulants, are reported in Table 1. For all the tested coagulants slight but significant differences were found between most of the proteolytic activities. This behaviour is generally observed independently of the milk clotting enzyme source.

Only the neutral protease activities of cheeses, made with the two liquid calf rennet–*Mucor miehei* mixtures, showed no significant differences. Activities of aminopeptidase and acid protease are more heterogeneous than those of neutral protease and acid phosphatase.

The enzymatic activities in Asiago pressato, which is a fast ripened cheese, are differentiated on the basis of their feature against age in days (Table 1). Both acid and neutral protease increase up to about 35% after 30 days of ripening. The activity of acid protease is 50% higher than that of the neutral one. This is in agreement with our previous data for Montasio cheese (SPETTOLI & ZAMORANI, 1985), and is probably due to the acidity level of the cheese approaching the optimum for acid protease activity. These results might indicate that acid rather than neutral protease activity has an important proteolytic action during Asiago pressato maturation which is in accordance with TRIEU-COUT and GRIPON (1982). The aminopeptidase activity also increases about 35% during the ripening time. This is in agreement with data of LAW and co-workers (1974) in Cheddar cheese extracts and of GOMEZ and co-workers (1988) in Spanish goat cheeses and partially with our previous data (SPETTOLI & ZAMORANI, 1985; ZAMORANI et al. 1988). The lactic acid bacteria multiply and produce their enzymes in young cheese during the first 24 h, afterwards enzyme synthesis stops and the cells slowly die (MEYER et al., 1989). Since the peptidases are exclusively intracellular, after bacterial lysis

they could be active in the following stages of ageing, notwithstanding their relatively higher optimum pH (LAW, 1984).

The acid phosphatase activity displays a slight but significant (about 7%) decrease in agreement with previous trials in Provolone Veneto and Asiago d'Allevo cheeses (SPETTOLI & ZAMORANI, 1985; ZAMORANI et al., 1988). It can be pointed out that the acid phosphatase behaves as the alkaline phos-

Table 1

Main effects of nine different commercial coagulants and ripening time on pH and enzymatic activities in Asiago pressato samples

Coagulant	Factors				
	pH	Acid protease	Neutral protease	Acid phosphatase	Amino-peptidase
Liquid microbial-animal blend	5.51	11.0 (cd)	7.7 (c)	255 (e)	1534 (e)
Liquid microbial-animal blend	5.52	12.7 (f)	8.2 (dc)	235 (d)	1228 (b)
Liquid animal	5.52	12.5 (f)	7.6 (c)	194 (ba)	1644 (f)
Liquid animal	5.53	10.7 (c)	6.5 (b)	198 (cb)	1417 (c)
Liquid animal	5.52	12.1 (ef)	7.8 (dc)	260 (e)	1475 (d)
Liquid microbial	5.49	8.6 (b)	6.3 (b)	236 (d)	1414 (c)
Powdered animal	5.30	7.5 (a)	4.2 (a)	204 (c)	941 (a)
Powdered animal	5.55	11.7 (de)	8.4 (d)	197 (cb)	1675 (f)
Powdered animal	5.53	11.4 (cde)	9.2 (e)	186 (a)	1494 (ed)
Ripening time (days)					
10	5.41 (a)	9.2 (a)	6.1 (a)	227 (c)	1232 (a)
20	5.53 (b)	11.1 (b)	7.6 (b)	217 (b)	1388 (b)
30	5.62 (c)	12.4 (c)	8.2 (c)	211 (a)	1654 (c)
Residual error of standard deviation	0.003	0.77	0.64	9.60	60.50
Degrees of freedom	16	81	81	108	162

The activities of acid and neutral protease, and aminopeptidase are expressed in terms of Δ O.D. $\times 10^{-3}$ h^{-1} g^{-1} of cheese at 37 °C at 280, 400, and 410 nm, respectively, in the assay conditions. The activity of acid phosphatase is expressed in terms of μg of p-nitrophenol $\times$ $\times 10^{-3}$ h^{-1} g^{-1} of cheese, in the assay conditions. a, b, c . . .: the average values not differing significantly at $P \leq 0.01$ probability level are marked with the same letter

phatase in sour milk cheese (SCHORMÜLLER, 1968) and in Camembert and Brie (VEISSEYRE, 1979). Our data are not discriminant for the origin of the enzyme. Therefore the acid phosphatase enzyme could also be released from the starter culture used i.e. *Streptococcus lactis* and *cremoris*. This possibility is reported by LARSEN and PARADA (1988), who suggested that this enzyme may eventually take part in proteolysis, allowing complete hydrolysis of casein phosphopeptides to phosphate residues, which are connected with curd texture.

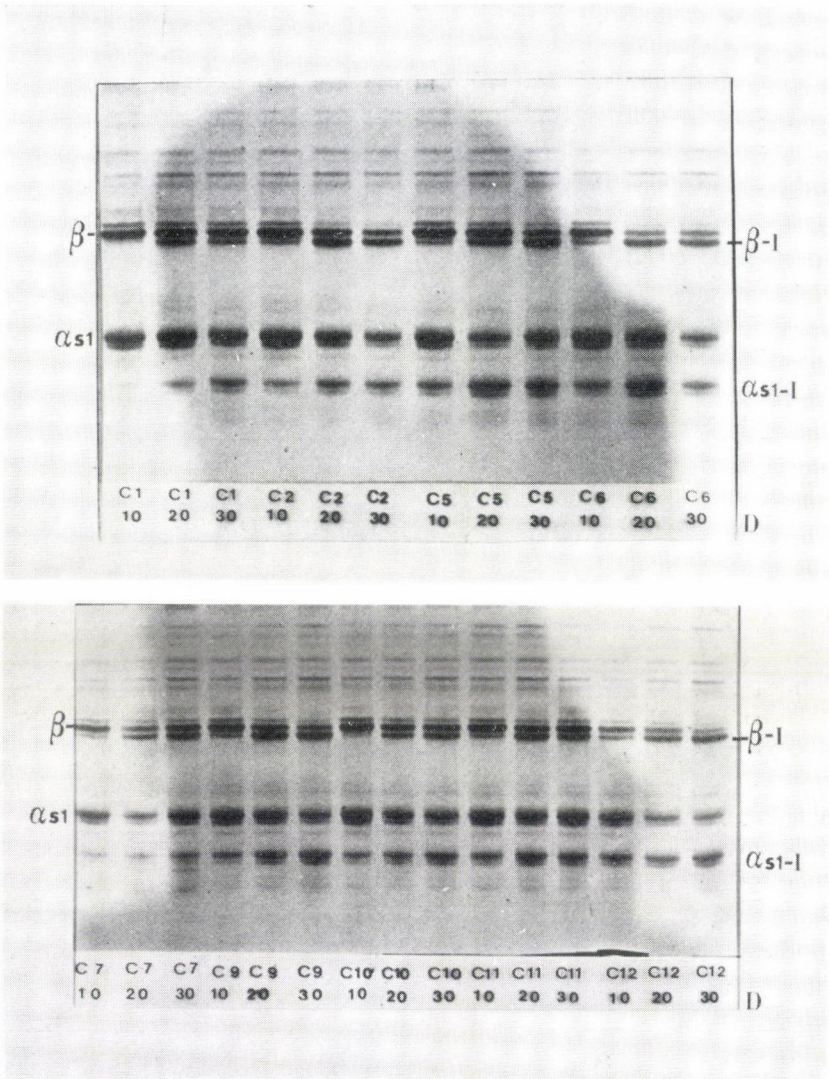

Fig. 1. Electrophoretic patterns of pH 4.6 insoluble nitrogen from Asiago pressato samples produced with various commercial coagulants during 10, 20 and 30 days of ripening. C1 = liquid microbial coagulant; C2 = liquid microbial-bovine blend coagulant; C5 = = liquid bovine coagulant; C6 = liquid bovine coagulant; C7 = liquid microbial-bovine blend coagulant; C9 = liquid bovine coagulant; C10 = powdered bovine coagulant; C11 = powdered bovine coagulant; C12 = powdered bovine coagulant

The electrophoretic patterns of the Asiago pressato samples display after 10 days of ageing hydrolysis in the β- and α_{s1}-casein fractions with the production of peptides having mobilities similar to those of β-I and α_{s1}-I caseins respectively (Fig. 1). The pH values of Asiago pressato ranged from 5.41 to

5.62 and the low salt concentration (about 2%) supports the idea that β-casein is degraded into β-I by the action of the coagulant during the first stage of cheese ripening as mentioned by GRAPPIN and co-workers (1985) and KAMALY and MARTH (1989). HOLMES and co-workers (1977) reported that 6% of the original rennet activity was still detectable in a pressed cheese, however the remaining chymosin can hydrolyse the protein in the curd (KAMALY & MARTH, 1989). Also, α_{s1}-I peptide, which is present at least in the early stages of ripening in all types of cheese (GRAPPIN et al., 1985; LAWRENCE at al., 1987), may be due to the rennet action in the initial breakdown of α_{s1}-casein in Asiago pressato. It has been observed that native milk proteinases, other than plasmin, could produce an α_{s1}-I like band in some cheeses, like Swiss-type cheese, where rennet probably was inactive (GRAPPIN et al., 1985). Because of the inability of *Streptococcus cremoris* proteinases to degrade α_{s1}-casein, it is unlikely that they take part in the early stages of ripening (LAWRENCE et al., 1987). As for the general protein patterns, a gradual protein breakdown from curd to 30-day-old samples is observed, being more marked from 10 to 20 days of ripening. There was no correlation between the electrophoteric patterns of the cheeses and the type of coagulant used for their manufacture.

3. Conclusions

It appears that coagulants of the same source but from different commercial firms produce Asiago pressato cheeses characterized by different proteolytic activities. Further investigations about pressed cheese proteolysis are needed to assess the specific roles of chymosin and plasmin, since both enzymes display similar behaviour during cheese ripening and contribution of plasmin is probably overshadowed by that chymosin (GRUFFERTY & FOX, 1988).

*

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INVESTIGATION INTO THE APPLICABILITY OF DIFFUSE REFLECTANCE AND TRANSMITTANCE TECHNOLOGY TO TOBACCO ANALYSIS

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To select the method for the quick determination of the total alkaloids, tar and reducing sugar in the Hungarian varieties of tobacco, the application of diffuse reflectance and transmittance technology was studied.

On the basis of results obtained in the 1100–2500 nm and the 400–1100 nm region using the reflectance and transmission techniques, it was established that the highest correlation coefficient and the lowest standard error of calibration can be achieved in the 1000–2500 nm region at reflectance mode.

To determine the relationship between the chemical composition data and optical data two types of statistics were used. Slightly higher correlation values were given by the partial least squares regression than by the derivative quotient regression. Using the partial least squares regression correlation coefficients of 0.987 for total alkaloids, 0.776 for tar and 0.975 for reducing sugar were obtained. It was possible to determine total alkaloids content within a standard error of calibration of $\pm$ 0.099 mass %, the tar content within $\pm$ 0.63 mass % and reducing sugar content within $\pm$ 1.099 mass %.

We concluded that the NIR technique ensures satisfactory accuracy for determination of total alkaloids and reducing sugar in Hungarian varieties of tobacco. But for the determination of tar further examination is needed.

Keywords: diffuse reflectance/transmittance spectroscopy, NIR technology, composition of tobacco

Determination of characteristic properties of tobacco is gaining increasing importance not only in the field of quality control, but in the field of processing optimization, too. To assure the mildness and aromaticity of smoke and at the same time to protect the health a great number of measuremetns are required during processing in tobacco industry.

Conventional chemical methods can hardly meet the increasing analytical demands, because they are time-consuming, the cumbersome procedures require special chemicals and several hours before test results are available. The quick, non-destructive physical methods offer the possibility of measuring the quality characteristics of raw material, semi-finished and finished products, and they can be connected to the processing lines.

Therefore, in recent years the interest of researchers has focused on the non-destructive optical methods. In 1968 MCCLURE already reported on the study of the spectral characteristics of tobacco. Later he and his co-workers

(McCLURE et al., 1977) used a scanning NIR spectrophotometer to determine the total reducing sugar in tobacco at different curing stages. Total alkaloids (HAMID et al., 1978), polyphenols (McCLURE & WILLIAMSON, 1982) were also investigated by NIR technology, obtaining high correlation between the chemical analyses data and reflectance spectra data.

In Hungary the tobacco industry is of great importance. According to the consumption data of cigarettes — which is the most popular tobacco product — Hungary is one of the countries, where the consumption is very high and the tendency did not show a decrease in 1980's.

In order to make possible the great number of analyses of the most important chemical constituents in tobacco, such as total alkaloids, tar and reducing sugar the possibility of application of diffuse reflectance and transmittance technology for this purpose was studied.

The near infrared diffuse reflectance (NIR) technology is widespread all over the world (WILLIAMS & NORRIS, 1987). It is also widely used in Hungary for quality control in different fields of food industry (KAFFKA et al., 1982; BIACS & KAFFKA, 1984; SZALÁNCZY & VÁRADI, 1984; KAFFKA, 1988). In the tobacco industry on-line methods have been intruduced for controlling the moisture content during the processing in several factories.

The aim of the present work was to choose the suitable methodology for Hungarian varieties of tobacco, to predict accuracy of analysis for the different components, to select the best form of the regression equation, to determine the parameters (characteristic wavelength, coefficients and constants), to examine the achievable accuracy by studies on the transformed reflection spectra and to use different statistics.

1. Materials and methods

Different varieties of tobacco grown in Hungary 119 samples were used in our investigation. The samples were collected from different districts of Hungary by the Tobacco Research Institute, Debrecen (Hungary). The samples were carefully prepared and analyzed using the traditional chemical methods at the Tobacco Research Institute. Grinding the samples was carried out by a coffee mill, which assured a fine, relatively homogeneous powder (appr. 0.2–0.4 mm particle diameter). Total alkaloids and tar contents were determined for all the 119 samples, the reducing sugar contents were measured for 70 samples. Determination of total alkaloids, tar and reducing sugar was performed using vapor distillation followed by spectrophotometry, pyrolisis followed by weighing and the Schoorl method, respectively.

Since the NIR technique is strictly correlative, the calibration samples were critically important, and the calibration samples should include all the

variability in composition (range of constituent values), particle size, sample treatment, etc. that might be encountered in any sample in practice to be measured. Taking these all into consideration, the tobacco samples were split into two sets. In the case of total alkaloids and tar we used 80 samples for calibration and 39 sample for validation. In the case of sugar the calibration set consisted of 47 samples and the validation set of 23 samples. The composition data of samples are listed in Table 1.

Table 1

Reference data for tobacco samples

Set	Alkoloids (mass%)	Tar (mass%)	Sugar (mass%)
Calibration A (80 samples)			
Mean	1.54	10.78	—
Standard deviation	0.53	1.18	—
High	3.57	16.58	—
Low	0.61	8.76	—
Validation A (39 samples)			
Mean	1.60	10.69	—
Standard deviation	0.67	1.09	—
High	3.52	13.96	—
Low	0.54	8.78	—
Calibration B (47 samples)			
Mean	1.44	10.93	4.8
Standard deviation	0.59	1.05	4.9
High	3.57	13.87	19.3
Low	0.61	8.76	0.2
Validation B (23 samples)			
Mean	1.60	10.99	4.4
Standard deviation	0.76	1.22	4.5
High	3.52	13.96	14.9
Low	0.54	8.78	0.2

The optical measurements were performed at USDA Instrumentation and Sensing Laboratory, Beltsville.

The near infrared reflectance properties of tobacco samples were measured with two types of scanning NIR spectrophotometers, namely with NIR System 6250 and NIR System 6500 (NIR System Inc., Silver Spring, MD, USA). 1100–2500 nm wavelength range was used in both instruments and 50 scans were averaged for each sample.

Using the NIR System 6500 we had the possibility to study the spectra in visible range and in reflectance and transmittance modes. In the reflectance

mode the 400–1100 nm range was examined, in transmittance mode, 700–1100 nm range was studied.

For reflectance measurements sample cups of 37 mm diameter, made in the Instrumentation and Sensing Laboratory were filled with tobacco powder. The cups had infrared transmitting quartz windows and were closed with a black rubber stopper backing. For transmittance measurements 0.500 g of sample were weighed into the cups obtaining approximately 1 mm sample thickness. Sample cups were not rotated in any of the modes.

The spectral data were recorded and stored by using an IBM compatible PC. Spectra were taken at 2 nm steps in the 1100–2500 nm range and at 4 nm steps in the 400–1100 nm range. The spectral data were stored as log (1/R) on floppy disk for further processing. The transformation of the R spectral curves to log (1/R) function gives a linear correlation with the concentration of a given measured component.

2. Results

The log (1/R) spectra in NIR range of tobacco samples chosen from the low and high concentration values of the constituents are shown in Figs. 1, 2 and 3. The difference in their curves is also illustrated in the Figures.

The measured optical data were evaluated with derivative quotients and partial least squares (PLS).

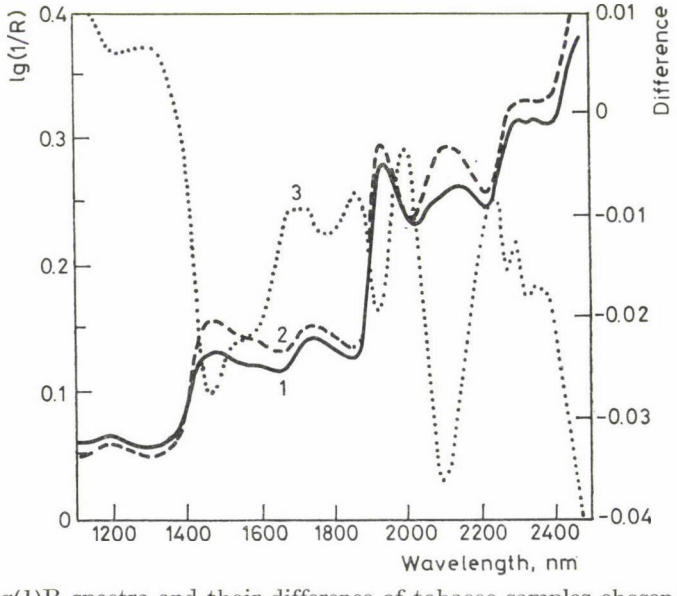

Fig. 1. The log(1)R spectra and their difference of tobacco samples chosen from the low and high concentration values of total alkaloids. 1: High alkaloid; 2: low alkaloid; 3: difference

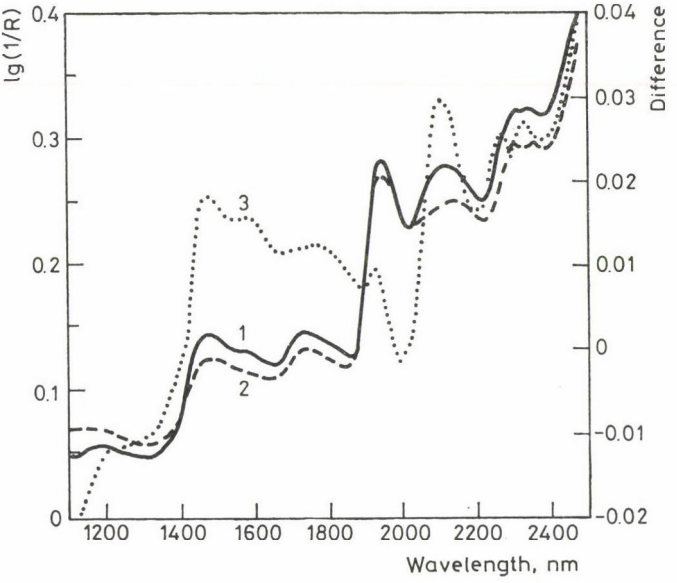

Fig. 2. The log(1/R) spectra and their difference of tobacco samples chosen from the low and high concentration values of tar. 1: High tar; 2: low tar; 3: difference

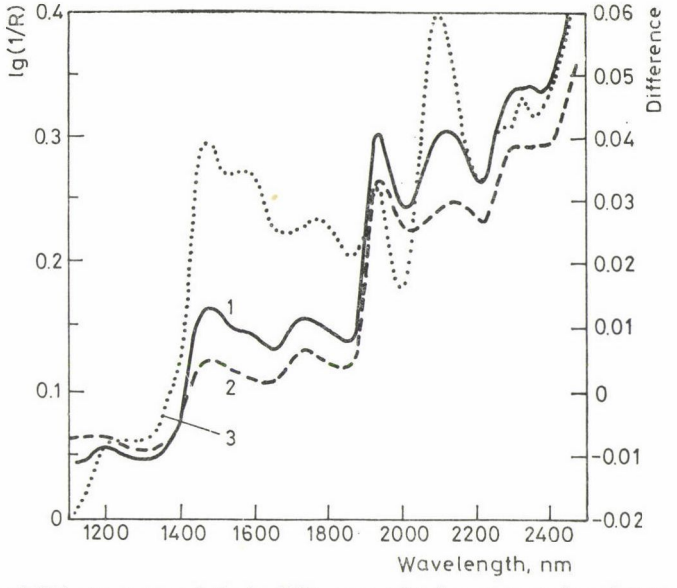

Fig. 3. The log(1/R) spectra and their difference of tobacco samples chosen from the low and high concentration values of sugar. 1: High sugar; 2: low sugar; 3: difference

2.1. Derivative quotient

Preliminary studies indicated that results could be improved by using second derivative of the log (1/R). Therefore, all data were analyzed by the use of the second derivative transformation at each wavelength point. Second derivatives are shown in Figs. 4, 5, 6.

The relationship between transformed spectral data and composition parameters was studied with the single term linear regression equation:

$$Y = a + bX$$

where Y stands for the composition parameters (total alkaloids, tar and reducing sugar), a and b are the regression constant and coefficient, respectively, and X is the quotient of two second derivatives of the log (1/R) spectra at wavelengths optimized for each composition parameter. The second derivative is defined here as

$$S''n = Sn + g - 2^* Sn + Sn - g$$

where Sn is the log (1/R) value at wavelength n, and g is a wavelength difference (gap).

As an illustration of the final stage of the optimization process, Fig. 7 shows the correlation plots of the numerator and denominator for the correlation to total alkaloids. The numerator plot is the correlation of the quotient

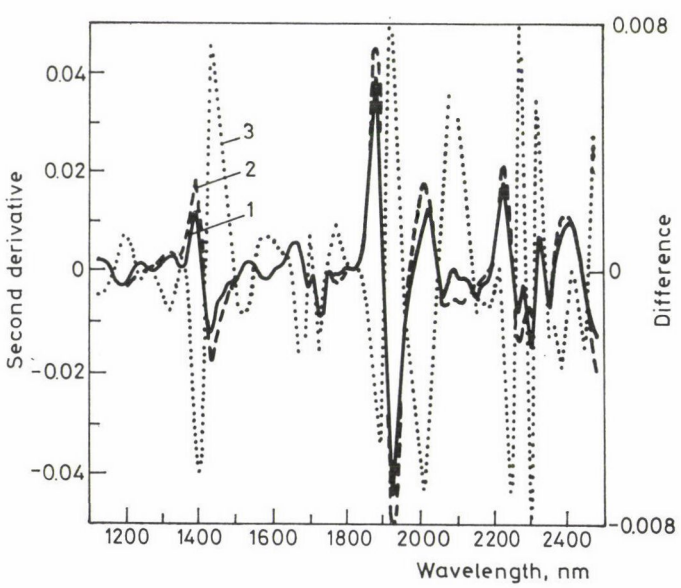

Fig. 4. The second derivatives of the log(1/R) spectra and their difference of tobacco samples chosen from the low and high concentration values of total alkaloids. 1: High alkaloid; 2: low alkaloid; 3: difference

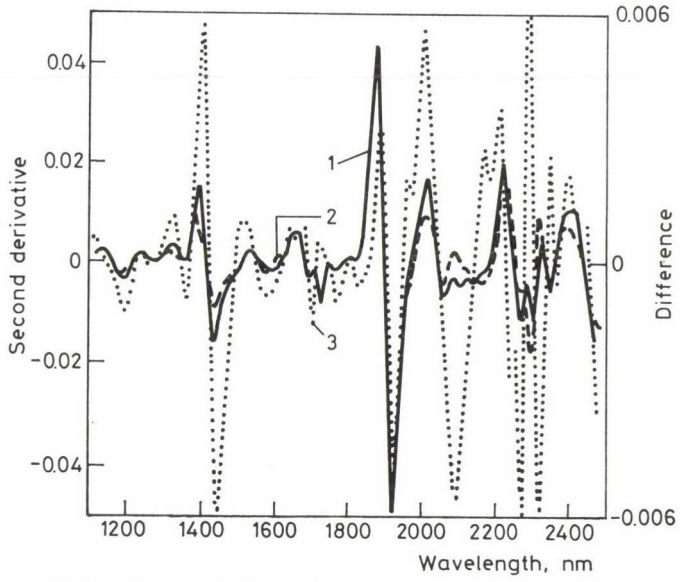

Fig. 5. The second derivatives of the log(1)R spectra and their difference of tobacco samples chosen from the low and high concentration values of tar. 1: High tar; 2: low tar; 3: difference

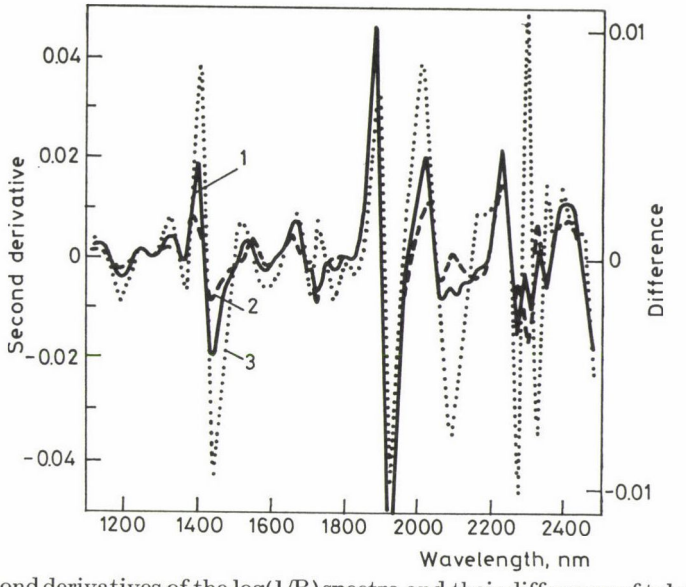

Fig. 6. The second derivatives of the log(1/R) spectra and their difference of tobacco samples chosen from the low and high concentration values of sugar. 1: High sugar; 2: low sugar; 3: difference

to total alkaloids when the denominator is fixed at 1668 nm and the numerator varies over the available wavelengths. In the denominator plot, the numerator is fixed at 1722 nm and the denominator varies. For all the three constituents the computer program determined the characteristic wavelengths, the gap for numerator and denominator, the correlation coefficients as well as the standard errors of calibration and validation. These data obtained in the near infrared range are summarized in Table 2.

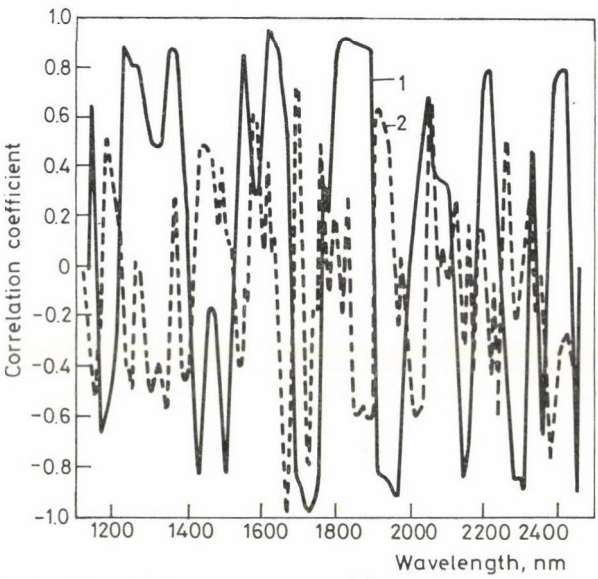

Fig. 7. The correlation plots of the numerator and denominator for determination of total alkaloids. 1: Numerator; 2: denominator

Because there was not a significant difference between the two instruments, only the data for the 6250 are shown.

In the visible and NIR ranges using the reflectance and the transmittance measurement modes there was no significant correlation between the optical and chemical data except for the sugar, where in reflectance mode 0.94 correlation coefficient was obtained at 986 nm and 500 nm wavelengths, in transmittance mode the correlation coefficient was 0.92 at 988 and 964 nm wavelegenths.

For testing the repeatability and reproducibility of measurements eight samples were chosen from the low and from the high concentration range of constituents. The repeatability was determined by taking the spectra of all the eight samples three times without repacking the sample cups. For the reproducibility measurements the sample cups were repacked for three spectra.

Table 2

Summary of linear regression analyses relating data from chemical analyses and values of the second derivative of log (1/R) curves at two characteristic wavelengths for the calibration set and for the validation set of tobacco samples, searching 1100 nm to 2500 nm

	For total alkaloids content	For tar content	For sugar content
Characteristic wavelength 1 (nm)	1722	1216	1454
Characteristic wavelength 2 (nm)	1668	1774	2350
Gap for numerator (nm)	20	22	3
Gap for denominator (nm)	7	34	15
Standard error of calibration (mass%)	0.138	0.699	1.01
Correlation coeff.	0.967	0.708	0.979
Standard error of validation (mass%)	0.147	0.821	1.322
BIAS (mass%)	—0.008	0.134	—0.048

Table 3

Reproducibility and repeatability for tobacco grounded samples expressed as the average of the standard deviations (S. D.) of the predicted data for total alkaloids, tar, reducing sugar using the second derivative quotients of log (1/R) curves at two characteristical wavelengths

Sample number	Repeatability S.D. mass%			Reproducibility S.D. mass%		
	Total alkaloids	Tar	Reducing sugar	Total alkaloids	Tar	Reducing sugar
10	0.007	0.039	0.150	0.012	0.039	0.216
17	0.003	0.008	0.035	0.001	0.032	0.071
21	0.008	0.011	0.091	0.010	0.027	0.406
28	0.019	0.005	0.092	0.025	0.035	0.146
113	0.005	0.014	0.264	0.001	0.037	0.251
114	0.019	0.061	0.165	0.010	0.033	0.073
123	0.008	0.046	0.082	0.011	0.085	0.294
306	0.006	0.046	0.057	0.008	0.006	0.353
Mean S.D. (mass%)	0.009	0.029	0.117	0.010	0.037	0.226
Mean conc. (mass%)	1.092	10.844	6.914	1.088	10.849	6.884

The repeatability and reproducibility were characterized by the average of the standard deviations of the predicted data of three scans for the eight samples. The data are shown in Table 3, where the mean of the predicted concentrations for the eight samples is also given.

Table 4

Summary of partial least squares regression analysis of tobacco samples, examining 1100 nm to 2500 nm

	For total alkaloids content	For tar content	For reducing sugar content
Number of factors	14	6	7
Standard error of calibration (mass%)	0.099	0.630	1.099
Correlation coeff.	0.987	0.776	0.975
Sample indicated as outlier	—	No. 32	—

2.2. Partial least squares

The PLS program from the NIR Systems NSAS program package was used. The number of factors for total alkaloids, tar and reducing sugar was determined by leverage method. 119 spectra were applied for determination of total alkaloids and tar, 70 spectra for sugar analysis. The results for the 1100–2500 nm range are summarized in Table 4.

As an illustration the result for total alkaloids is graphed in Fig. 8. In the visible range, similar to the derivative quotient method significant correlations were found between the chemical and optical data of tobacco for total alkaloids and for tar. For sugar determination using 7 factors we got correlation coefficient of 0.9450 and standard error of 1.69 mass% in transmittance mode. Correlation coefficient of 0.9743 and standard error of 1.148 mass% were obtained with 9 factors in reflectance measurements.

To grove the results the cross validation was applied.

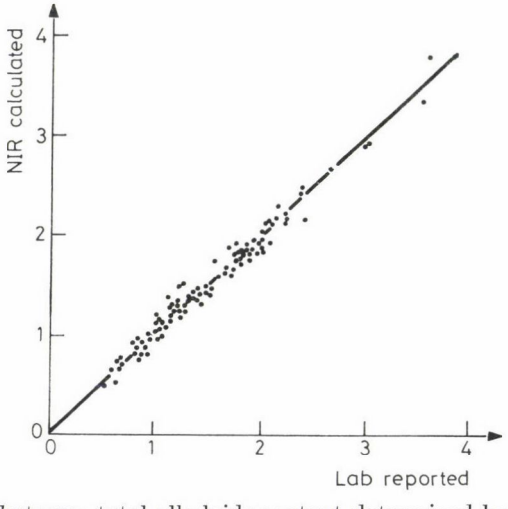

Fig. 8. Relationship between total alkaloids content determined by traditional chemical analysis and predicted value by NIR using the PLS regression analysis

3. Conclusions

Comparing the results obtained at different wavelength range by employing reflectance and transmittance spectroscopy, it can be stated, that the highest accuracy can be achieved for the three constituents of tobacco — such as total alkaloids, tar, reducing sugar — in the range of 1100–2500 nm using the reflectance mode.

Our experiments showed that the single-term prediction equation — applying the ratio of second derivative of log (1/R) measured at two characteristic wavelengths — ensures satisfactory accuracy for the determination of total alkaloids and reducing sugar. The correlation coefficient of 0.96 for total alkaloids and 0.97 for reducing sugar were obtained. It was possible to determine total alkaloids content within a standard error of calibration of $\pm$ 0.138 mass%, reducing sugar content within $\pm$ 1.01 mass%. The data of validation proved to be similar.

In the case of tar for improving the results it is needed to modify the sample distribution and to control the accuracy of chemical analysis. It can be assumed that the procedure of pyrolysis does not ensure high accurate results to the NIR calibration.

The application of the partial least squares regression analysis resulted in the best correlation coefficients and best standard error for all the three constituents, although the improvement over derivatives quotients was not so significant. The correlation coefficients of 0.987 for total alkaloids, 0.975 for reducing sugar and 0.776 for tar were obtained. The determinations were carried out for total alkaloids content within a standard error of calibration of $\pm$ 0.099 mass%, for reducing sugar content within $\pm$ 0.975 mass% and for tar content within $\pm$ 0.63 mass%.

The transmission technology proved to be not a suitable method for the characterization of ground tobacco samples, because of the high scattering and the low absorbance of the ground samples.

Summarizing, the high correlation coefficients and the good repeatability and reproducibility of NIR measurements indicates that the NIR technique has the potential for use in rapid determination of total alkaloids and reducing sugar in tobacco.

*

Thanks are due to the co-workers of the USDA-BARC Instrumentation and Sensing Laboratory, to Ms. JOYCE SHAFFER for her conscientious assistance in performing measurements and data processing, to Dr. STEPHEN DELWICHE for assistance in instrument modification and data processing. The authors would also like to express their thanks to Ms. PIROSKA KOCSIS at Tobacco Research Institute for supplying the tobacco samples.

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ERRATUM

STERILIZATION OF SPICES AND VEGETABLE SEASONINGS BY GAMMA RADIATION

G. LESCANO, P. NARVAIZ and E. KAIRIYAMA

Vol. 20, No 3—4. p. 240.

Table 5

Sensory evaluation of control and 30 kGy spices and vegetable seasonings on the 1st and 6th storage months. Significant differences between control and 30 kGy samples ($P \leq 0.05$)

Samples	Colour	
	1st month	6th month
Ginger	yes*	no
Cayenne pepper	yes*	yes*
Turmeric	no	no
Onion powder	yes**	yes**
Garlic powder	yes**	yes**

no : not significantly different from control
yes: significantly different from control
* : slightly lighter
** : slightly darker

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DETERMINATION OF BENZO(A)PYRENE IN THE LIQUID SMOKE PREPARATIONS UTP-1 BY HIGH PRESSURE LIQUID CHROMATOGRAPHY AND CONFIRMATION BY GAS CHROMATOGRAPHY — MASS SPECTROMETRY

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A very fast method for determination of benzo(a)pyrene in the liquid smoke preparations UTP-1 was elaborated.

After alkalization, samples were several times extracted by cyclohexane. Combined cyclohexane extracts were not only concentrated and dried, but purified by solid-phase extraction as well. Finally benzo(a)pyrene concentrations were determined by high pressure liquid chromatography with selective fluorescence detection and results were confirmed by gas chromatography–mass spectrometry using the selected ion monitoring technique. The results have shown that the method developed is operatively performable, and offering more than 97% yielding capacity of added benzo(a)pyrene standard. On the base of obtained results it was found, that benzo(a)pyrene concentrations in meat products flavoured by liquid smoke preparations UTP-1 are significantly lower in comparison to meat products flavoured by traditional ways.

Keywords: benzo(a)pyrene, gas chromatography, high pressure liquid chromatography, liquid smoke preparations

Flavouring of meat products by derivatives of thermal destruction of wood with the purpose of obtaining certain typical organoleptic properties is one of the frequently employed finalizing technological steps in the production of foods.

However, in addition to sensory active compounds (phenol derivatives, carbonyls), the smoking process involves the formation of compounds harmful to human health, viz. polycyclic aromatic hydrocarbons (PAHs), the quantity and composition of which is influenced by several factors (POTTHAST, 1978; TÓTH, 1983).

The most important compound of PAHs is benzo(a)pyrene (BaP). This one is a typical product of wood thermal destruction, although it constitutes only between 1 and 20% of the total carcinogenic PAHs (ANDELMAN & SUESS, 1970). Because BaP can be found everywhere as products of burning and has an expressive cancerogenic effect on live organism (HOWARD & FAZIO, 1980), legislative measures prescribes to carry out the control of BaP contents

in those types of food products or additives where the risk of PAHs contamination is posing an objective hazard factor e.g. smoked meat products.

The determination of PAHs or BaP by gas chromatography with a flame ionization detector as well as mass spectrometer has been described in many papers (VASSILAROS & STOKER, 1982; LAWRENCE & WEBER, 1984; MAGA, 1986; BIERI & GREAVES, 1987).

The use of flame ionization detectors is hampered by the need for very thorough clean-up procedures, with the accompanying risk of severe losses and possible misidentifications. For this reason, the use of a mass spectrometer (MS) in the mode of selected ion monitoring (SIM) makes it possible to simplify time-consuming clean-up procedures (TUOMINEN et al., 1986).

The technique of high pressure liquid chromatography (HPLC) offers promise as an effective method for separation and analysis of PAHs or BaP, especially. In all references a chemically modified silica gel has been used as a stationary phase for separation of PAHs using ultraviolet as well as selective fluorescence detection (ZOOCOLILLO et al., 1984; KRUIJF et al. 1987; STIJVE & HISCHENHUBER, 1987; SIMKO, 1991).

This work is aimed at determination of BaP in the liquid smoke preparations UTP-1, the new wood flavour made in Czechoslovakia. BaP was determined by HPLC method with fluorescence detection and the results were confirmed by GC–MS method using SIM technique.

1. Materials and methods

1.1. Materials

The liquid smoke preparations (LSP) UTP-1 were made in Slovenské lucobné závody, state enterprise, Czechoslovakia.

Standard of BaP was obtained from Supelco, Switzerland (Cat. No. 4–8564).

All organic solvents, acetonitrile "HPLC grade" (Fluka), both methanol and cyclohexane "analyzed reagent grade" (Lachema) were before application redistilled in a glass distillation unit with a rectification column.

Silica carts filled with Separon SGX C 18 (40 μm nominal particle size) were obtained from Tessek, Ltd (Cat. No. 421 310013).

1.2. Sample preparation

Hundred g of LSP were transferred into a 500 cm^3 separatory funnel, 150 cm^3 of KOH solution (20% weight) were added, and the funnel contents shaken during 2 min. Next, 50 cm^3 of cyclohexane were added and shaken thoroughly, after which the aqueous layer was left to separate, and subsequently removed.

The cyclohexane extraction was repeated three more times and the combined cyclohexane layers were washed twice with 100 cm^3 water. Subsequently the cyclohexane extract was dried by passing through anhydrous Na_2SO_4 and evaporated in a rotary vacuum evaporator at 45 °C to near dryness. The residue was dissolved in 1 cm^3 of methanol. The silica cart was washed with 10 cm^3 of methanol, and dried with 3 cm^3 of air. Then, the methanol solution of the sample residue was applied to the top of the silica cart, and the PAHs fraction was eluted with 3 cm^3 of methanol. The eluate was collected and evaporated to the volume of 1 cm^3 again. From the sample thus prepared, 15 μl were used for analyses by HPLC, or 10 μl for analyses by GC—MS.

1.3. HPLC conditions

HPLC was performed isocratically on a Separon SGX C 18 column (the column consisted of two series of connected columns, 5 μm, 15 cm × 3 mm i.d., Tessek Ltd, Cat. No. 901 300013), The separation of PAH proceeded at ambient temperature by using a mobile phase (acetonitrile–water 3 : 1 v/v) with a flow rate of 1.15 $cm^3\ min^{-1}$. The effluent from the column was directed to a Perkin-Elmer LS-2B fluorescence detector. The excitation wavelength was set to 310 nm and emission wavelength to 410 nm.

1.4. GC—MS conditions

A Hewlett-Packard HP 5985 A gas chromatograph with a mass detector was used, operating under the following conditions: using a splitless injector, a sample was injected into the equipment, and a 25 m long fused silica capillary column (0.2 mm i.d.) was used for separation. The column (HP-5) was crosslinked with 5% phenylmethylsilicone by WCOT technique resulting in the 0.33 μm thick film. As a carrier gas helium was used at pressure 195.557 kPa and the following temperature programmed: 50 °C for 0.5 min, then increasing to 180 °C at 30 °C min^{-1} gradient, followed by further increase to 300 °C at 7 °C min^{-1} gradient. The temperature of ion source was 200 °C, the open-split interface 250 °C, an analyser 200 °C; dwelling time 200 min, multiplier voltage 2 kV, an electron impact energy 70 eV. The typical ion fragments of BaP-252.3; 250.2; 126.1 were monitored by a HP 21 MX—E datasystem.

2. Results

2.1. Extraction and preliminary purification

The limiting factor of any used method is its yielding capacity, predetermining the applicability of the method itself. From this aspect, multiple cyclohexane extraction has proved very useful since excellent yields were

obtained at the level of 1 μg of added BaP standard. The percentual yields of BaP obtained with the use of cyclohexane extraction are summed up in Table 1.

Table 1

Extraction yields of added BaP from the liquid smoke preparation UTP-1 at level 1 μg of BaP

Extraction No.	Yield (%)
1	98.2
2	96.9
3	97.7
4	97.0
5	97.2
Average yield	97.4

First of all, of course, the yielding capacity of solid phase extraction (SPE) had been determined. The best results were obtained at 3 cm^3 of methanol as follows from Table 2.

Table 2

Solid phase extraction yields of BaP standard solution at concentration 1 μg BaP per cm^3 of methanol

Volume of eluent (cm^3)	Yield (%)
0.5	0.0
1.0	0.0
1.5	17.5
2.0	49.2
2.5	89.7
3.0	99.6

Note: Yields are average of triplicates

2.2. Identification of BaP by HPLC method

The BaP identification was carried out by adding the standard BaPs solution (1 μg BaP in 1 cm^3 of methanol) to an analyzed sample. Figure 1 shows the chromatographic record obtained from the LSP (a) while (b) shows chromatographic record of the same sample with added BaP standard solution.

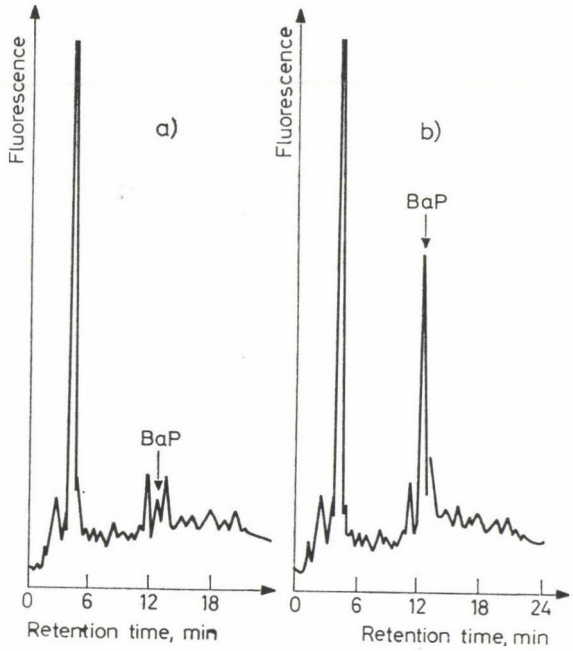

Fig. 1. HPLC chromatograms of the sample. Liquid smoke preparation UTP-1; chromatographic record of the sample itself (a) and chromatographic record of the same sample with added BaP standard solution (b)

2.3. Identification of BaP by GC—MS method

The elution time of BaP was determined on the base of analyses of standard BaP solution. During this time, the characteristic mass spectrum of BaP was obtained (Fig. 2). After having been compared to the data in Library of mass spectra, three of the most intensive masses (252.3; 250.2; 126.1) were scanned by SIM technique (Fig. 3).

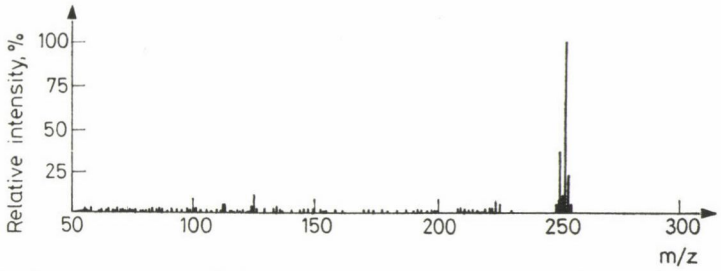

Fig. 2. GC—MS spectrum of BaP. m/z: the ratio of a mass to charge of ions analysed

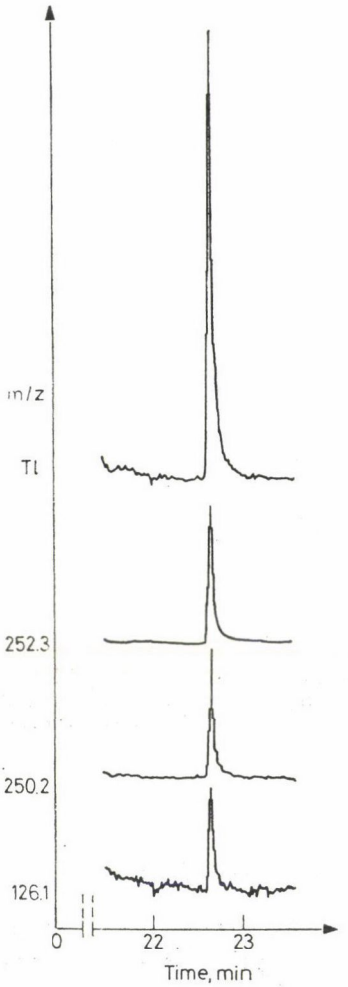

Fig. 3. GC—MS chromatographic record of the sample. Liquid smoke preparation UTP-1 obtained by SIM technique. Chosen masses 252.3; 250.2; 126.1 were scanned in the field of BaP standard elution time. m/z: the ratio of a mass to charge of ions analyzed; TI: the sum of all masses of ions scanned (Total Ion chromatogram)

3. Discussion

Based upon the obtained results, the BaP contents were calculated in three different LSP UTP-1. These results are summed up in Table 3, where column A shows the results obtained by HPLC method, and column B shows the results of the same samples obtained by GC—MS method. As follows from these results, the differences between results obtained by HPLC and GC—MS method are statistically insignificant. The results have also shown that the BaP contents have not exceeded the value of 10 μg per kg. This value is

considered as the maximum allowable limit for using of LSP in food products by Expert Commission for Additive of FAO/WHO. In addition to this, the actual quantity of a LSP necessary for creating an optimum organoleptic profile of meat products is a significant parameter. The LSP UTP-1 are added in amounts corresponding to 100 mg of phenolic compounds, i.e. 10 g of the LSP to 1 kg of a flavoured meat product (SIMKO et al., 1989). It follows from this, that the actual BaP contents in flavoured products are decreased by the order of two, and correspond to values shown in Table 3, column C. These

Table 3

BaP contents determined by chromatographic methods and BaP contents calculated for 1 kg of flavoured meat products

Liquid smoke preparation UTP-1 No.	A (μg per kg)	B (μg per kg)	C (μg per kg)
1	0.83	0.79	$7{,}9-8.3\times10^{-3}$
2	0.57	0.60	$5{,}7-6.0\times10^{-3}$
3	0.31	0.27	$2.7-3.1\times10^{-3}$

Column A : BaP contents determined by HPLC method,
column B : BaP contents determined by GC—MS method,
column C : BaP contents calculated for 1 kg of flavoured meat product,
values in column A and B are averages of duplicates, the duplicate sample values of determinations did not exceed the level of $\pm0.7\%$ standard deviation

values are virtually negligible in comparison to the BaP contents found in traditionally smoked meat products (STIJVE & HISCHEHUBER, 1987; SIMKO et al., 1991). On the other hand, small amounts of BaP were found in another LSP (TÓTH & BLAAS, 1972; SILVESTER, 1980).

4. Conclusion

Based on obtained results, the elaborated method of sample preparation as well as BaP determination by HPLC method were found very fast, enabling for a check of BaP contents in the LSP UTP-1, because at given conditions the limit of detection of BaP concentration was found to be 0.03 μg per kg.

In addition to this, the BaP contents found in foods flavoured with LSP UTP-1 is, in practice, negligible in comparison to foods flavoured by traditional ways of smoking.

Finally, the method itself is suitable for fast assessment of BaP contents in these types of food additives.

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EFFECT OF WATER ACTIVITY ON THE KINETICS OF DESTRUCTION OF *ESCHERICHIA COLI* BY CHEMICALS

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The dependence of the rate of destruction by chemicals on water activity was studied in experiments carried out with *Escherichia coli*. The disinfectant applied was a quaternary ammonium base of widespread application in the food industry whose effectiveness was determined in the water activity range of 0.874–0.995.

In the course of the evaluation of the experimental results by covariance analysis no significant difference was found between the concentration exponents determined at various water activities, the concentration exponent valid for the whole range being $n = 3.2 \pm 0.4$.

A mathematical model with two independent variables was established for describing the dependence of the death rate coefficient on disinfectant concentration and water activity. The correlation coefficient between the calculated values and those determined experimentally was: $r = 0.9727$.

Keywords: water activity, rate of destruction, concentration of disinfectant, concentration exponent

When describing the killing of microorganisms by disinfectants, similarly to thermal destruction processes, a first order chemical reaction serves as a suitable basis.

The differential equation describing the concentration of surviving cells at constant temperature and disinfectant concentration is as follows:

$$\frac{dN}{dt} = -kN \tag{1}$$

where N = the concentration of living cells
k = the specific rate of destruction

Reaction (1) as proposed by CHICK (1908) has been in general use till today for describing thermal or chemical destruction of microorganisms, in spite of the fact that, in many instances, deviations from first order kinetics can be observed (RUSSEL, 1971; KING et al., 1979; SOUSA et al., 1987; ENGEL & TEUBER, 1991).

In order to characterize the connection between the death rate coefficient and the concentration of disinfectant, the concentration exponent introduced by WATSON (1908) is used, which is determined from the destruction rates measured at different concentrations of chemical.

$$n = \frac{\log k_2 - \log k_1}{\log c_2 - \log c_1} \tag{2}$$

where n = the concentration exponent

k_1 and k_2 are the the rate constants of destruction determined at concentrations c_1 and c_2.

Determination of the concentration exponent is essential in the practical use of disinfectants because it allows calculation of the decrease in activity caused by dilution.

The mean concentration exponents of some types of disinfectants reported in the literature have been summarized in Table 1.

Table 1

Concentration exponents of some disinfectants

Group of chemicals	Average conc. exponent	Reference
Alcohols	10	BEAN (1967)
Phenols	6	BEAN (1967)
Quaternary ammonium bases	1	BEAN (1967)
Mercuric chloride	1	HUGO (1976)
Formaldehyde	1	HUGO (1976)
Chlorine dioxide	1	BENARDE et al. (1967)
Hypochlorous acid	1	ODLAUG (1981)

The efficiencies of the various types of disinfectants might to a great extent be influenced by environmental factors (pH, temperature, water activity, organic and anorganic substances).

Ample data are available in relation to the effect of pH (BEAN, 1967; HUGO, 1971; BLOCK, 1977; JONES, 1987).

In order to characterize the temperature dependence of microbial death caused by disinfectants, in analogy to chemical reactions generally use is made of activation energy (BENARDE et al., 1967) or Q_{10} value (BEAN, 1967). The Q_{10} values of disinfectants of similar types are nearly identical: for formaldehyde 1.5, for phenols 3–5, for aliphatic alcohols 30–50 (BEAN, 1967).

As to the effect of water activity, an important environmental factor from the aspect of the application of disinfectants in the food industry, data from the literature are available in an indirectly. Authors mostly analyze the effects of substances of various types (fats, proteins, inorganic salts, etc.) without referring to the water activity of the medium (BEAN, 1967; JONES, 1987).

A relationship describing the effect of water activity on thermal destruction of microorganisms has been suggested by MOSER (1988):

$$k = k_0 a_w \exp - \frac{E_A \cdot a_w}{RT} \tag{3}$$

where k = rate coefficient,

k_0 and E_A are constants calculated from experimental results,

a_w = water activity.

Relationship (3) can be applied also for isothermal reactions and might serve for establishing a relationship between water activity and the rate of microbe destruction caused by chemicals.

This investigation includes a study of the dependence on concentration and water activity of a quaternary ammonium base disinfectant generally applied in the food industry, in relation to destruction of *Escherichia coli*. On the basis of our results a mathematical model has been established, which is suitable for describing, within the range studied, the dependence of the rate constant of destruction on chemical concentration and water activity.

1. Materials and methods

1.1. Disinfectant

The disinfectant used was a quaternary ammonium base compound in liquid phase, marketed in Hungary under the trade name of Polacid Forte. The use concentration recommended for spray-disinfection, disinfection of bottle washing machines and hand disinfection is 0.1–0.2% aqueous solution. In our experiments the liquid as sold was considered 100%, and concentrations of the chemical were related to this value.

1.2. Test microorganism

The strain *Escherichia coli* NCA1M B 00200 from the National Collection of Agricultural and Industrial Microorganisms (of Hungary) has been used throughout the experiments.

The strain was maintained in the lyophilized state. The medium used for culturing was composed as follows:

peptone	5 g
yeast extract	2.5 g
glucose	1fg
agar	29 g
distilled water	1000 g

The incubation temperature was 37 °C, sterilization 121 °C 15 min, pH = 6.8.

1.3. Experimental method

Investigations were carried out at room temperature (22–24 °C), at various combinations of water activity and disinfectant concentration. The water activity was controlled by glycerol, as recorded in Table 2.

Table 2

Glycerol concentrations adjusted for the experiments

(Calculated on the base of HANDBOOK OF CHEMISTRY AND PHYSICS, 52nd ed., 1971–1972)

Water activity	g glycerol per 100 g solution
0.995	2.5
0.956	17.7
0.928	25.8
0.900	32.3
0.874	37.5

For the destruction experiments the surface growth of 4 tubes of 24 h agar slants were washed into 200 cm^3 aqueous glycerol of appropriate water activity, then the suspension was mixed for 10–15 min to achieve osmotic equilibration.

Numbers of viable microorganisms were determined by plate counting. Colonies were counted after incubation for 48 h at 37 °C. A sterile physiological salt solution was used as diluent. In order to neutralize residual disinfectant, 3% Tween 80 and 0.3% lecithin were added to the first dilution series.

The logarithms of colony counts were plotted against time, and the rate constants of destruction calculated from the slopes of the survival curves thus obtained.

By plotting the logarithms of destruction rate constants versus the logarithms of the corresponding concentrations of the disinfectant, the slope of the resulting straight line gives the value of the concentration exponent.

2. Results and discussion

The destruction rate constants as a function of water activity and disinfectant concentration are summarized in Table 3.

The data in Table 3 were evaluated by covariance analysis according to SVÁB (1981) in order to determine, whether changes in water activity affected the value of the concentration exponent to a significant extent. The results are given in Table 4.

Table 3

Destruction rate coefficients (k) *of* Escherichia coli *as a function of water activity* (a_w) *and disinfectant concentration* (c)

(Disinfectant: Quaterner ammonium base Polacid Forte, pH = 6.1, temperature = 22–24 °C)

a_w	c (mg dm^{-3})	lg c	k (min^{-1})	lg k
0.995	80	1.903	1.12	0.050
	100	2.000	2.52	0.400
	110	2.041	4.92	0.692
0.956	140	2.146	1.43	0.155
	160	2.204	2.44	0.388
	180	2.255	3.50	0.544
0.928	140	2.146	0.302	−0.520
	160	2.204	0.523	−0.282
	200	2.301	1.108	0.044
0.900	300	2.477	0.601	−0.221
	500	2.699	2.69	0.430
	600	2.778	4.41	0.645
0.874	400	2.602	0.259	−0.586
	600	2.778	0.967	−0.015
	800	2.903	2.42	0.383

Table 4

Covariance analysis of the lg c − *lg* k *values summarized in Table 3*

(SVÁB, 1981)

Factor	SSQ	DF	V	F	F_s
Deviation from linear regression					
Total for treatments	7.484	5	1.50		
Within treatment jointly	3.085	9	3.43		
Between treatments	0.893	3	0.30	86**	3.8
Hypothesis					
Common equation	2.207	8	0.29	192**	4.8
Common slopes	2.337	4	5.84	3.9	5.2

SSQ: Sum of squares of deviations
DF: Degrees of freedom
V: Variance
F: Calculated F value
F_{95}: Critical F value at 95% significance level
** Difference at 99% significance level
Calculated value of common slope with the 95% confidence interval: 3.25 ± 0.38

From a comparison of the calculated and critical values of F in Table 4, it can be concluded that, in the range investigated, a_w does not affect the concentration exponent.

The common concentration exponent calculated by covariance analysis taking into account its 95% confidence interval amounted to

$$n = 3.2 \pm 0.4$$

which is significantly higher than the mean value of $n = 1$ shown in Table 1 for quaternary ammonium compunds.

The effect of disinfectant concentration on destruction rate, as a function of water activity, is shown in Fig. 1. The figure shows the curves of common slope calculated as a result of covariance analysis.

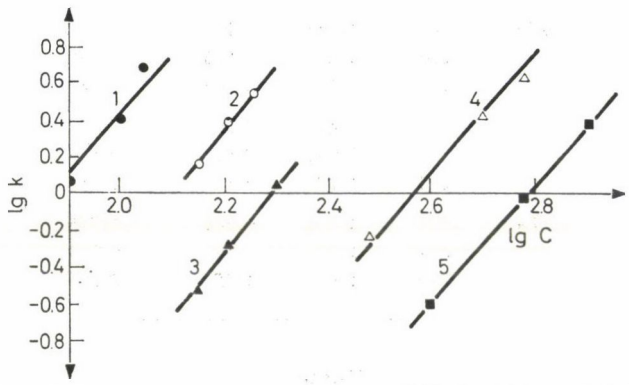

Fig. 1. Logarithm of the destruction rate constant of *Escherichia coli* (lg k) as a function of the logarithm of disinfectant concentration (lg c) at different water activities (1: a_w = = 0.995, 2: $a_w = 0.956$, 3: $a_w = 0.928$, 4: $a_w = 0.900$, 5: $a_w = 0.874$). Disinfectant: quaternary ammonium base Polacid Forte

In order to establish the dependence of the destruction rate constant on disinfectant concentration and water activity, a linear regression model with two independent variables was fitted to the experimental data according to SVÁB (1981). The mathematical relationship applied was as follows:

$$\lg k = a + b_1 \lg c + b_2 \lg a_w \tag{4}$$

The results of regression analysis were, together with the 95% confidence intervals of the regression coefficients, as follows:

$a = -5.14$
$b_1 = 2.90 \pm 0.47$
$b_2 = 48.8 \pm 7.3$
correlation coefficient: r = 0.9727
number of data pairs: $n = 15$

The standardized regression coefficients (path-coefficients) expressing the relative effect of concentration and water activity are:

$$b_1 \text{ standard} = 2.27$$
$$b_2 \text{ standard} = 2.45$$

As the two standardized coefficients are nearly identical, it can be concluded that, in the range investigated, the destruction rate constant is equally sensitive to changes in disinfectant concentration and in water activity.

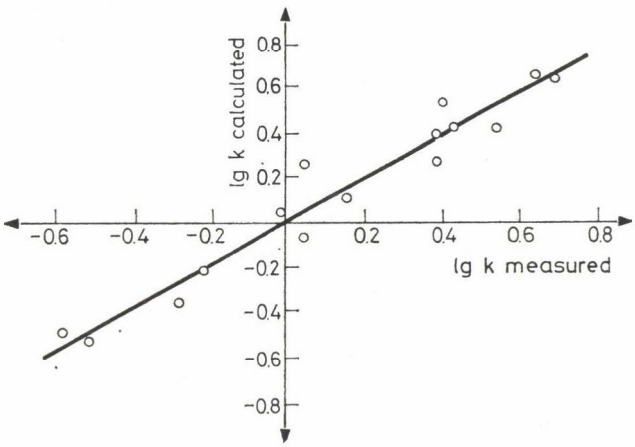

Fig. 2. Relationship between the measured and calculated values of the logarithm of the destruction rate constants

Comparing the values of the common concentration exponent $n = 3.2 \pm 0\ 40$ and $B_1 = 2.9 \pm 0.47$ characteristics of disinfectant concentration, it can be inferred that no significant difference exists.

The logarithmic destruction rate constants calculated with the mathemathical model applied are shown, as a function of the experimentally determined values, in Fig. 2.

The mathematical-statistical characteristics of the linear relationship between the values measured and calculated are as follows:

slope	$= 0.946 \pm 0.135$
intercept	$= 0.007 \pm 0.056$
standard deviation around the curve	$= 0.095$
correlation coefficient	$= 0.9727$
number of data pairs	$= 15$

Taking into account the established standard deviation, the destruction rate constant can be calculated with the mathemathical model (4), in the range investigated, with relatively high accuracy.

Starting from the value of 0.095, characteristic of the estimation of the logarithm of the rate constant, the variation coefficient characteristic of the destruction rate constant results to be $\pm$ 24%.

The mathematical-statistical analysis concluded that the water activity has a significant effect on the efficiency of disinfectant. For example decreasing the water activity from 0.995 to 0.874, the same efficiency (destruction rate) needs nearly ten-fold increase in disinfectant concentration. Because the concentration exponent seems to be independent of the water activity, the effect of the dilution of disinfectant remained the same in the range investigated.

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RADIATION PRESERVATION OF WHITE POMFRET *(STROMATEUS CINEREUS)* IN TOMATO SAUCE

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A method for the radiation preservation of white pomfret *(Stromateus cinereus)* in tomato sauce is described which enables the storage of fish at ambient temperature (28 ± 3 °C) for a period of six months. The fish was devoid of microbial contamination and was acceptable as determined by organoleptic evaluation. The product with low pH could be stabilized with a dose of 5 kGy. Biochemical and bacteriological evaluation confirmed the storage stability of the product. The process offers a safe and simple method to preserve white pomfret in a "heat and serve" form.

Keywords: white pomfret, radiation preservation

A radurization process for the refrigerated storage of white pomfret *(Stromateus cinereus)*, a medium fatty fish, has been developed earlier in this laboratory (KAMAT & KUMTA, 1972). It has been reported that the mild discoloration and generation of off-odors observed in radiation sterilized sea foods could be effectively suppressed if the fishes were dipped in tomato sauce prior to exposure to sterilization doses of gamma irradiation (KARDASHEV, 1966). A method developed on the basis of the above observations is described here, specifically for the preservation of white pomfret. Advantage of the combination process involving radiation and heat is taken in the present method which also employs an effective management of pH whith the incorporation of tomato sauce. This procedure permits storage of white pomfret at ambient temperature (28 ± 3 °C) upto six months without spoilage.

1. Materials and methods

1.1. Preparation of fish samples

Freshly caught white pomfrets were purchased from a local market. They were brought in ice to the laboratory, cleaned with water, beheaded, eviscerated and cut into pieces of 5 cm length. The fillets were washed thoroughly with water and stored in a cold room (0–2 °C) in ice before giving further treatments.

1.2. Preparation of tomato sauce

Tomato sauce was prepared using ingredients listed in Table 1. Chopped onions were deep fried in edible oil till the contents turned brown. Onion in oil was made into a paste by grinding. Tomato puree was boiled for 10 min with salt, sugar and the onion paste along with oil. Spices were added one by one (Table 1) and the boiling was continued for further 5 min under continuous stirring. Acetic acid was finally added (Table 1) to the hot sauce.

Table 1

Ingredients of tomato sauce
for 5 kg of fish, pH 4.3

Ingredients	Weight (g)
Tomato puree (80% water)	700
Onions	170
Oil	35
Sugar	80
Salt	10
Coriander	0.35
Clove	0.35
Black pepper	0.35
White pepper	0.35
Ginger	0.15
Cinnamon	0.15
Acetic acid	13.00

1.3. Dip treatment

White pomfret fillets were dipped for 2 min in chilled brine (20% NaCl) and drained completely.

1.4. Steam cooking and canning

White pomfret fillets (100 g) were placed in 2272 cm^3 metallic C-enamel cans and steamed in an exhaust box for 20 min. The external temperature of the exhaust box was 103 °C. This treatment resulted in inactivating the enzymes and reduced the initial bacterial load. Steamed fillets (100 g) were filled in 2272 cm^3 metallic C-enamel cans separately and fully covered with hot tomato sauce.

The various treatments which were used in the experiment are as follows:

— Fresh white pomfret fillets without tomato sauce or vinegar;
— Steam cooked fillets without tomato sauce or vinegar;
— Fillets, steam cooked and filled up with vinegar (4% acetic acid);
— Fillets, steam cooked and filled up with tomato sauce (55% by weight)

The net weight of each can was maintained at 220 g. All the cans including those not filled with tomato sauce or vinegar were exhausted for 10 min in steam and sealed under vacuum (22 sec) in a vacuum sealer. Sealed cans were stored at 0–2 °C for two days prior to irradiation to permit ingredients of the sauce to penetrate the muscle.

1.5. Irradiation

A batch of packed cans (consisting of cans of fish with tomato sauce and the control cans) were subjected to a dose of 5 kGy in a Cobalt 60 Package Irradiator (Atomic Energy of Canada Ltd.) at a dose rate of 0.045 kGy min^{-1} and stored at ambient temperature (28 $\pm$ 3 °C).

1.6. Assessment of quality

During the storage period of upto 60 days the samples were subjected at regular intervals to sensory evaluation, bacterial assessment and measurement of chemical qualities. After sixty days of storage, the samples (with tomato sauce) were analysed for sensory attributes only.

Canned fish samples were warmed in boiling water for 5 min and served to six trained panel members for evaluation of quality in terms of taste, texture, odour and appearance based on a 5 point hedonic scale.

1.7. pH determination

The acidity of fish samples was measured by recording the pH of 50% fish homogenate.

1.8. Moisture determination

Moisture in fish and sauce was determined by drying 10 g portion of the sample to a constant weight at 105 °C.

1.9. Indices of freshness

Total bacterial count (TBC) and total volatile basic nitrogen (TVBN) were determined as described by GORE and KUMTA (1970). Free fatty acids (FFA) were estimated as described by KAMAT and KUMTA (1972). Total volatile acid (TVA) was determined by the method of FARBER and FERRO (1956).

2. Results and discussion

A heat-radiation combination method which also incorporates acidity provided by the tomato sauce enhances the organoleptic acceptability of the product and ensures its microbiological safety. The process differs from conven-

tional canning in that there is no involvement of heat sterilization as the heating is done in exhaust box for 20 min. Table 2 lists the sensory evaluation score of steam cooked pomfrets in tomato sauce irradiated at 5 kGy and stored at ambient temperature (28 ± 3 °C) for six months. The product was rated acceptable by the panelists during this period as seen from the overall score.

Table 2

Sensory evaluation of steam cooked pomfrets (irradiated with 5 kGy) in tomato sauce, stored at 28 ± 3 °C

Storage time days	Appearance	Odor	Texture	Taste	Overall score
1	4.33	4.33	4.00	4.35	4.25
7	4.08	4.16	3.63	4.28	4.04
15	4.33	3.83	3.50	4.25	3.98
28	4.00	4.16	3.08	4.15	3.85
60	3.83	3.83	3.00	4.00	3.67
120	3.33	3.83	3.33	3.80	3.57
150	3.05	3.75	3.25	3.45	3.38
180	3.00	3.27	3.33	3.25	3.21

Taste panel scores were based on a 5 point hedonic scale wherein 5 = like very much, 4 = = like, 3 = neither like nor dislike, 2 = dislike, 1 = dislike very much. The values represent the average of 3 independent experiments. The variation in sensory evaluation values were within the range of 3.7%.

The amenability of irradiated fish in sauce to room temperature storage for extended periods of six months could be explained on the following lines. Steam cooking of fish in cans to an internal temperature of 80 °C inactivates enzymes and reduces the initial bacterial load. Low pH (pH 4.3–4.8) of vinegar and tomato sauce prevents the growth of surviving bacteria. Any acid tolerant surviving bacteria and contaminating molds subsequent to heat treatment were inactivated by irradiation at 5 kGy. An upper limit of 5 kGy was chosen in order to achieve freedom from microorganisms including the pathogenic ones. A pH be less than 5.0 prevailing throughout the storage period (Table 3) provides safety against botulism hazard, since *Clostridium botulinum* spores are reported to be incapable of germinating and producing toxin at these pH conditions (EKLUND & POYSKY, 1970).

Table 4 shows results on the bacterial profile of fish subjected to different treatments and stored at ambient temperature (28 ± 3 °C). Fresh and cooked pomfrets in the absence of vinegar or sauce showed bacterial proliferation during storage leading to spoilage within a week. Cooked pomfrets in vinegar permitted slow growth of bacteria. Irradiation at 5 kGy caused re-

Table 3

Changes in the pH and moisture content of pomfret in tomato sauce during storage at 28 ± 3 °C

Storage time (days)	pH of 50% homogenate		Moisture in fish (%)		Moisture in sauce (%)	
	0 kGy	5 kGy	0 kGy	5 kGy	0 kGy	5 kGy
1	4.30	4.30	65.67	62.95	79.40	77.27
7	4.65	4.35	61.79	63.11	76.74	78.68
15	4.65	4.45	62.70	65.00	78.15	79.25
28	(S)	4.60	(S)	66.45	(S)	82.13
60	(S)	4.15	(S)	65.00	(S)	77.90

The pH value is the average of 5 determinations on duplicate samples upto day 7. The rest represents the average of 3 separate experiments. Moisture value was assessed only once. (S) indicates spoiled. The variation in pH values was within the range of 2.3%.

Table 4

Changes in total bacterial counts of pomfrets packed under different conditions and stored at ambient temperature (28 ± 3 °C)

Fish sample	Dose (kGy)	Storage time (days) 1	7	15	28	60
		Bacterical count (log N g^{-1})				
Fresh	0	7.62	(S)	(S)	(S)	(S)
(Control)	5	6.34	7.52(S)	(S)	(S)	(S)
Steam cooked	0	6.95	7.72(S)	(S)	(S)	(S)
(Control)	5	< 1.00	7.15(S)	(S)	(S)	(S)
Steam cooked	0	< 1.00	1.78	7.55(S)	(S)	(S)
fish in vinegar	5	< 1.00	< 1.00	< 1.00	7.32(S)	(S)
Steam cooked	0	< 1.00	< 1.00	< 1.00	7.53(S)	(S)
fish in tomato sauce	5	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00

Counts were obtained for 1 c.c. of 10% fish homogenate (10g fish in 90 c.c. saline). The initial count (0 day) of fresh fish were 4.30 (0 kGy) and 1.11 (5 kGy). TBC values represent the average of 3 separate determinations in triplicate. (S) indicates spoiled. The variation in TBC values were within the range of 1.8%.

duction of bacterial counts from these samples and resulted in the storage stability upto two weeks. On the contrary, cooked pomfrets in tomato sauce irradiated at 5 kGy did not show significant bacterial counts during storage for 60 days.

Table 5 includes the data on the indices of freshness of cooked pomfret in tomato sauce stored at ambient temperature (28 ± 3 °C). Total volatile basic nitrogen (TVBN) an index of freshness of fish, increased from 11.63 to 19.36 mg per 100 g in 60 days. It should be noted that despite some increase in the TVBN even after irradiation at 5 kGy, the product is not spoiled. An

Table 5

Changes in TVBN, TVA numbers and FFA in cooked pomfrets in tomato sauce stored at 28 ± 3 °C

Storage time (days)	TVBN		TVA		FFA	
	0 kGy	5 kGy	0 kGy	5 kGy	0 kGy	5 kGy
1	11.27	11.63	28.80	24.10	2.20	2.80
7	12.49	13.36	24.40	22.40	2.40	2.82
15	14.95	15.81	20.80	22.00	2.50	2.60
28	(S)	14.82	(S)	25.60	(S)	2.95
60	(S)	19.36	(S)	24.80	(S)	3.60

The values for fish samples were analysed from the homogenate which is made from the whole content of a can. It includes fish and tomato sauce. TVBN values are expressed in terms of mg nitrogen per 100 of homogenate. TVA are expressed as cm^3 of 0.01*N* NaOH for 100 g of homogenate. FFA are expressed as micromoles of oleic acid g^{-1} of homogenate. The values represent the average of 3 separate determinations. (S) indicates spoiled. The variations in TVBN, TVA and FFA were within 3.5%, 4.6% and 1.3%, respectively.

increase in TVBN content in irradiated sample is not abnormal since it has already been demonstrated that the linear relationship between TVBN and radiation dose exists only upto a dose level of 2.5 kGy (DOKE et al., 1976). However, another index of spoilage, total volatile acids (TVA) did not change significantly during the entire period of storage. Radiation induced oxidative changes measured in terms of release of free fatty acids (FFA) are also shown in Table 5. The red color of tomato sauce interfered with the analysis of 2-thio--barbituric acid (TBA) value. Irradiation at 5 kGy produced a slight increase in FFA values initially but they remained almost constant during the entire period of storage.

In the absence of enzymes and bacteria, biochemical changes mainly arising from these factors are minimal and therefore only oxidative changes could be implicated in the degradation of quality of the present product. Radiation induced oxidative changes are suppressed in pomfret-in-tomato sauce possibly due to high acidity as suggested by KARDASHEV (1966). The chemical changes that could occur in fish in a reaction with acetic acid leading to discoloration are masked by tomato sauce. The radiation induced odors are also similarly masked by tomato sauce.

Textural changes occurring in pomfret-in-tomato sauce are minimal. However, during the later period of storage (5 months) some dryness was noticed by sensory panel members. Nevertheless, these changes do not lead to unacceptability of the product upto six months. Thus, the above process offers safe and simple method to preserve white pomfret in a "heat and serve" form and the process offers potential avenues of application to other fishery products. Although packaging in cans has been adopted in this investigations,

suitability of other packaging methods could be explored to attain similar product stability.

*

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PHYSICOCHEMICAL AND NUTRITIONAL CHARACTERISTICS OF *MICHELIA CHAMPACA* SEED OIL

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Michelia champaca seeds contain 10.4% resin and 30.9% dark yellow coloured semi-solid fat. The alcohol-alkali refining and bleaching with fullers earth and carbon reduced the high acid value, high unsaponifiable matter and dark colour of the crude seed oil. The fatty acid composition (wt%) of the refined seed oil was: palmitic 24.2, stearic 5.8, oleic 66.1 and linoleic 3.9. Nutritional quality of the refined seed oil was evaluated by feeding the seed oil and groundnut oil as control to rats at 10% level for 4 weeks. The growth performance, digestibility, feed efficiency ratio, serum lipids and histopathological findings were satisfactory for the refined seed oil.

Keywords: *Michelia champaca*, non-traditional plant seeds, fatty acid composition, nutritional study

Shortage for edible oils in India emphasizes the need to explore the minor and lesser known unconventional oils (LAKSHMINARAYANA, 1987). Studies on some seed oils of nontraditional plants have been recently reported from our laboratory. As a part of our survey on newer oilseeds, the physicochemical and nutritional properties of *Michelia champaca* seed oil are reported here.

Michelia champaca (syn. *Michelia aurantiaca*; fam. Magnoliaceae) is a moderate sized evergreen tree with smooth grey bark and narrow leaves somewhat resembling that of mango. The trees are much cultivated in gardens, roadsides and in the vicinity of temples for their strongly scented flower. The flowers appear in April and thereafter at intervals throughout the hot weather and rains. The fruits consist of long clusters of capsules, from which the brown coloured seeds ultimately hang out on long cords. The fruit is said to be edible.

1. Materials and methods

1.1. Extraction and processing of the seed oil

M. champaca seeds contain resin and to estimate it, a weighted amount of ground seeds were gently extracted with a minimum quantity of chilled 95% ethanol. The extract was filtered. The ground seeds were further washed

twice with chilled ethanol. About 50 cm^3 chilled ethanol was used (for the total procedure) for 10 g of the seed powder. Ethanol-washed seeds were air dried and weighed. Resin content of the seeds was equal to the difference in weight on chilled ethanol extraction. The ethanol-washed seeds were extracted with n-hexane in a Soxhlet distillation apparatus for 72 h and evaporation of the solvent was done under vacuum. The seed fat was refined with alcoholic caustic soda at the concentrations of 0.4% and 0.2% in two stages and then bleached with 1.5% fullers earth and 0.3% activated carbon.

1.2. Analytical methods

Physicochemical constants of the refined seed oil were determined by conventional of the AMERICAN OIL CHEMISTS' SOCIETY (1973). Methyl esters were prepared by transesterification of the oil using methods methanol containing 1.0% sodium methoxide after diazomethane esterification of the free fatty acids. Methyl esters were analyzed by gas liquid chromatography (GLC) using a 15% DEGS column on chromosorb WHMDS (MAITY & MANDAL, 1990). Infrared (IR) spectra of the oil and its methyl esters were taken using a Beckman Model 221 IR spectrophotometer in KBr disc and ultraviolet (UV) absorption was taken in CCl_4 on a Beckman 26 UV-visible spectrophotometer. Thin layer chromatography (TLC) of the oil and its methyl esters were done separately on 0.25 mm Silica Gel G coated glass plates using n-hexane, diethyl ether and acetic acid (79 : 20 : 1; v/v) and spraying with concentrated sulphuric acid. The methyl esters of castor and sal seed oils were used for reference.

1.3. Nutritional evaluation

For nutritional evaluation of the refined seed oil, 12 male albino rats of local strain (inbred in our laboratory), age 20–24 days and weighing about 50–60 g were divided into two groups of 6 animals each and individually caged. Animals in both groups were fed a stock standard diet containing 10% oil (MANDAL et al.,1982). Other ingredients of the diets were (g per 100 g): casein 10, starch 50, sucrose 20, cellulose powder 5, salt mixture 4 and vitamin mixture 1. One group of animals were fed on 10% refined groundnut oil diet and another on 10% refined *M. champaca* seed oil diet. The animals received their respective diet and water ad libitum for 4 weeks. Food-intakes were recorded daily and body weight weekly. Feed efficiency ratio (FER) which represents the weight gain for unit food intake was calculated. Digestibility of the oil was determined by estimating the oil intake and oil excreted through urine and faeces (RAGHURAMULU et al., 1983). At the end of the experimental period, the animals were sacrificed, blood was collected and total

lipids (FOLCH et al., 1957), phospholipids (FISKE & SUBBAROW, 1925), free fatty acids (HEINDEL et al., 1974) and cholesterol (SPERRY & WEBB, 1950) were determined. Histopathological examination was done in the organs like liver, heart, kidney and reproductive organs under the microscope.

2. Results

M. champaca seeds contain resin at a level of 10.4%. The oil content of the seeds was fairly high (30.9%). The UV and IR spectra showed no conjugated or trans unsaturation, respectively. TLC analysis did not show the presence of any epoxy, hydroxy or cyclopropene fatty acids in the seed oil. Data on physicochemical constants and fatty acid composition of the refined seed oil are given in Table 1. The physicochemical constants were normal. GLC analysis of the methyl esters showed that the seed oil is rich in oleic acid (66.1%) followed by palmitic acid (24.2%). Other fatty acids present in the seed oil were stearic (5.8%) and linoleic (3.9%). The crude oil has a dark yellow colour, high unsaponifiable matter (8.4%) and high acid value (52.9%). The alcohol-alkali refining and bleaching of the crude seed oil reduced the visible dark yellow colour to light yellow, acid value from 52.6 to 8.4 and unsaponifiable matter from 8.4% to 3.6%. The final product after refining was obtained in a yield of 82%. The data obtained from the feeding study are shown in Table 2. It was observed that the rats fed 10% refined seed oil diet for 4 weeks showed a body weight gain which was about 93.6% of that obtained with groundnut oil. The mean value for FER of the refined seed oil was 22.4

Table 1

Physicochemical characteristics and fatty acid composition of the refined seed oil of Michelia champaca

Physicochemical constants:	
Refractive index at 25 °C	1.4647
Acid value	8.4
Saponification value	197
Iodine value	60.1
Unsaponifiable matter (%)	3.6
Fatty acids (wt%):	
Palmitic	24.2
Stearic	5.8
Oleic	66.1
Linoleic	3.9

The data are mean values of three determinations

Table 2

Nutritional indices and serum lipids of rats fed diets containing refined groundnut oil and refined Michelia champaca *seed oil for 4 weeks*

Parameters studied	Diet containing 10% groundnut oil		Diet containing 10% seed oil	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Body weight gain (g) for 4 weeks	48.3	4.6	45.2	3.9
FER	24.3	2.4	22.4	2.6
Digestibility of fat (%)	94		90	
Serum				
Total lipids (mg per 1000 cm^3)	1254	82	1284	52
Phospholipids (mg per 1000 cm^3)	782	36	741	34
Cholesterol (mg per 1000 cm^3)	628	43	683	38
Free fatty acid (mM per 1000 cm^3)	0.32	0.02	0.34	0.03

$\bar{x}$: mean value from six rats; $\pm s$: standard deviation, FER (feed efficiency ratio): body weight gain per food intake $\times$ 100. All values are non-significant when compared between two groups

and 24.3 for refined groundnut oil. The digestibility of the seed oil was 90% compared to 94% for groundnut oil. The concentrations of serum lipids of the rats fed the refined seed oil were within normal range and showed no significant differences from those obtained for the animals fed groundnut oil. Histopathological examination did not reveal any abnormalities in any organ of the animals fed refined seed oil.

3. Conclusions

3.1. Chemical composition and processing

The *M. champaca* seeds contain 10% resin which need to be further investigated. Oil content of the seed is fairly high (30.9%) which indicates that it may be exploited commercially. The high unsaponifiable matter (8.4%), the high acid value (52.6) and dark yellow colour of the crude seed oil were some of the undesirable factors which were easily reduced by alcohol-alkali refining and subsequent bleaching. The refined seed oil has low unsaponifiable matter (3.6%) and moderate acid value (8.4). It was also devoid of any objectionable colour and odour. The loss upon refining was only 18%, a figure which is not very high for the non-traditional seed oilds. Physicochemi-

cal characteristics and fatty acid composition of the refined seed oil are comparable to those of some common edible vegetable oils. The seed oil contains no unusual constituent or unusual fatty acid. Thus, the chemical composition of the refined seed oil suggests that it may be non-toxic and may be used for edible purposes.

3.2. Nutritional quality of the refined seed oil

It has been found that the rats fed 10% refined seed oil diet for 4 weeks showed a good growth performance. The value for FER and digestibility for the refined seed oil was also satisfactory. Serum lipids and histopathological findings were also normal in animals fed the diet containing refined seed oil. The rats fed the seed oil did not show behavioural abnormalities or any toxic side reaction. Thus, it may be concluded that the refined seed oil of *M. champaca* is non-toxic to lower animals at least at the level of 10% and its nutritional performances indicate the possibility of its exploitation as edible oil.

*

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THE STRATEGY OF EFFICIENT PRODUCTION OF BREWER'S YEAST BIOMASS

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The effect of preaeration, addition of unsaturated fatty acids and the most important fermentation parameters — sugar concentration and aeration level on brewer's yeast production — was investigated in synthetic and malt germ medium.

Increased aeration level (500 $1h^{-1}$) has a beneficial effect on growth rate, but the lower intensity resulted in higher protein content.

Yeast growth at different maltose concentrations underline the effect of glucose on brewer's yeast, causing catabolite repression of the respiratory enzymes.

Supplementation of fermentation broth with linseed oil caused a drastic decrease in lag period. The unsaturated oil is co-utilized which results in a higher growth rate at 0.5% linseed oil concentration.

Preaeration of the inoculum is also effective in the reduction of lag period without any effect on the growth rate.

Keywords: biomass production, brewer's yeast, aeration effect, yeast growth determination

Brewer's yeast, the biocatalyst of beer production, has also importance as a highly valuable protein and vitamin B source. For the brewing process the inoculum (pitching yeast) is not always a freshly prepared yeast suspension but often a 2–4 times used, sedimented and washed yeast. As brewing is an almost anaerobic fermentation the yeast cells are inhibited in the synthesis of unsaturated fatty acids, important components of the cell wall. Several authors reported on the beneficial effect of sterols under anaerobic conditions.

Ergosterol, sitestol and calciferol were equally active under aerobic conditions in cotton-plugged culture flasks. Brewer's yeast shows relatively long generation time but especially the extended lag phase has a negative influence on biomass productivity.

Aeration intensity has a significant effect on yeast production not only on biomass yield but also on the methionine content (HALÁSZ et al., 1989). The importance of sugar concentration in case of Crabtree positive yeasts is well-known, especially the long-term adaptation, which involves the repression of respiratory enzymes in aerobic batch cultures (VAN URK et al., 1990). Catabolic repression not only reduces the yield coefficient but influences also the quality (fermentation activity, shelf-life, etc.) of the yeast.

The aim of this work was to investigate the effect of aeration intensity, maltose content, medium composition on brewer's yeast fermentation and the possibility to reduce the lag period by adding growth promoting agent or pretreatment of the inoculum by aeration.

1. Materials and methods

1.1. Yeast strain

Brewer's yeast, production strain of Hungarian Brewery, Böcs.

1.2. Culture media

Two different media were used, synthetic liquid culture medium and a malt germ medium.

Composition of the individual culture media:

Synthetic medium

$MgSO_4$	0.1 g dm^{-3}
KH_2PO_4	0.14 g dm^{-3}
Na_2HPO_4	0.8 g dm^{-3}
NaCl	1.0 g dm^{-3}
$(NH_4)_2SO_4$	5.0 g dm^{-3} pH was adjusted to 4.5.

The medium was completed with maltose as carbon source in the concentration range of 0.5–2.0%. The medium was sterilized by autoclaving up to 0.516 bar.

Malt germ medium

Fifty g dry malted barley germ were suspended in 100 cm^3 water and autoclaved up to 0.516 bar. After filtration the clear solution was autoclaved again up to 0.516 bar and complemented with maltose (0.1–0.5%). pH of the medium was adjusted to 4.5.

1.3. Preparation of yeast culture inoculum

Fresh or frozen stored (at −13 °C) brewer's yeast slurry was preaerated for 2 h in a column fermenter if not otherwise mentioned. Yeast concentration of fermentation broth was adjusted to OD between 0.15–0.20.

1.4. Determination of protein content

Kjel-Foss automatic type instrument was used using 0.50 g dried yeast biomass. Mean values were calculated from two parallel determinations.

1.5. Determination of yeast growth

The growth of yeast was followed by measuring the optical density of the medium and by determination of the dry weight of cell content of an aliquot sample. Cells were harvested by centrifugation, washed, resuspended in 10 cm^3 distilled water. Suspension was transferred into glass dishes and dried at 105 °C. Mean value was calculated from two parallel determinations.

1.6. Method of yeast propagation

Column fermentor: 6 cm diameter, double wall glass columns of 400 cm^3 capacity were used. Filtered air was introduced from the bottom of the column through a glass filter (G4). Temperature was regulated by connecting the columns to a constant temperature circulating water bath. The pH was adjusted and maintained at pH 4.5 by the addition of 2.5% NH_4OH solution. Aeration rates of 250, 750 and 1250 $l\,h^{-1}\,l^{-1}$ medium were applied. Fermentations were carried out in two parallels and three replications.

1.7. Mathematical statistical evaluation of the results

Experimental data were evaluated by Student t-test and variance analysis, respectively.

2. Results

2.1. The effect of aeration intensity on growth rate and protein content of brewer's yeast

Yeast growth rate expressed as $\frac{\Delta\,OD}{\Delta\,t}$ is significantly influenced by the aeration intensity in both media (Fig. 1 and Table 1).

Highest growth rates were observed at 1200 $l\,h^{-1}\,l^{-1}$ aeration levels and there is no difference between the synthetic and malt germ media when maltose content in both is equally 0.5%. This finding is in agreement with the observation of KASSIM and and HALÁSZ (1989) who investigated the effect of mineral medium and date syrup medium on yeast growth.

Best protein values however resulted from fermentations with lower (750 $l\,h^{-1}\,l^{-1}$) aeration intensity. Also in this respect there was no significant difference between the two media investigated.

2.2. Effect of maltose concentration on the growth of brewer's yeast

Fermentation experiments were made at 750 $l\,h^{-1}\,l^{-1}$ aeration intensity. The effect of maltose was investigated at three concentration levels (0.5, 1.0 and 2.0%) in case of synthetic medium. As it can be seen in Fig. 2 and Table 2,

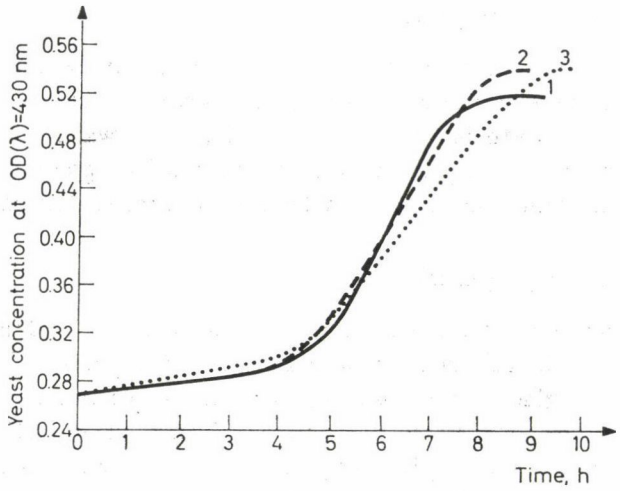

Fig. 1. Effect of aeration intensity on the growth of brewer's yeast propagated in synthetic medium. Inoculum: frozen stored, preaerated yeast maltose concentration 0.5%. 1: aeration 250 $1h^{-1}1^{-1}$, tg $\alpha = 0.06$; 2: aeration 750 $1h^{-1}1^{-1}$, tg $\alpha = 0.07$; 3: aeration 1250 $1h^{-1}1^{-1}$, tg $\alpha = 0.09$

Table 1

Effect of aeration rate on growth rate $\left(\frac{\Delta OD}{\Delta t}\right)$ *of brewer's yeast propagated in column fermentor in 0.5% maltose containing medium*

Aeration ($1h^{-1}1^{-1}$)	Synthetic medium			Malt germ medium		
	250	750	1250	250	750	1250
$\frac{\Delta OD}{\Delta t}$	0.05	0.07	0.09	0.04	0.08	0.09
Protein % of dry weight	44	47	41	48	51	46

growth rate was highest when 0.5% maltose was added to the synthetic medium. At 2% maltose content the stationary phase started in the 8th hour of propagation. At the lower sugar concentrations higher yield biomass content was achieved. This phenomenon can be explained by the respirofermentative metabolism of the Crabtree positive yeast wich results in a lower yield and in that case also a lower mean growth rate at 750 $1h^{-1}1^{-1}$ aeration intensity. We investigated also the effect of lower maltose levels in malt germ medium. As shown by the growth curves in Fig 3 at maltose contents of 0.1 and 0.3% the lag period was shorter which also underlines the effect of glucose on brewer's yeast causing catabolite repression of the respiratory enzymes (KEEVIL et al., 1979).

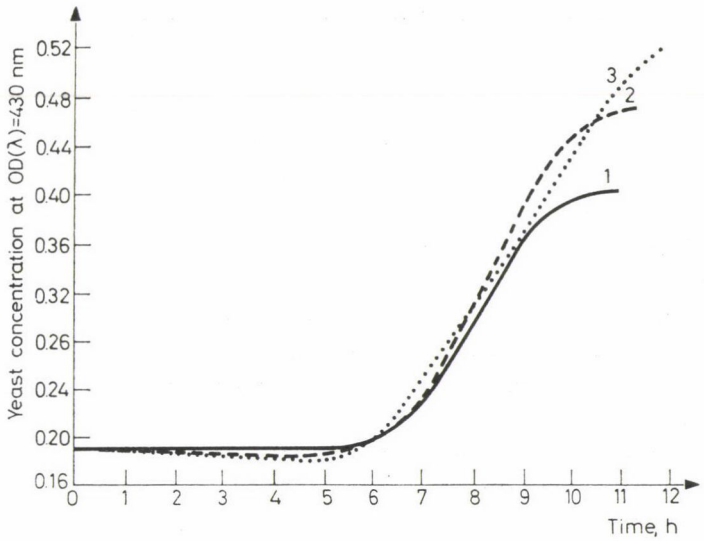

Fig. 2. Effect of sugar concentration on the growth of brewer's yeast, propagated in synthetic medium. (Inoculum: frozen stored yeast without preaeration, aeration intensity: 750 $1h^{-1}l^{-1}$). 1: 0.5% maltose, tg $\alpha = 0.07$, 2: 1.0% maltose, tg $\alpha = 0.08$; 3: 2.0% maltose tg $\alpha = 0.03$

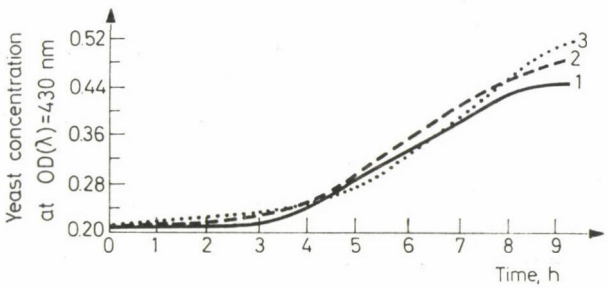

Fig. 3. Effect of maltose concentration on yeast growth (propagated in malt germ medium, aeration: 750 $1h^{-1}l^{-1}$). 1: 0.1% maltose, tg $\alpha = 0.04$; 2: 0.3% maltose, tg $\alpha = 0.08$; 3: 0.5% maltose, tg $\alpha = 0.07$

Table 2

Effect of maltose concentration on the growth rate $\left(\frac{\Delta OD}{\Delta t}\right)$ *of brewer's yeast propagated in column fermentor at 750 l* h^{-1} *l* $^{-1}$ *aeration level*

	Synthetic medium			Malt germ medium		
	0.5	1.0	2.0	0.1	0.3	0.5
	maltose content (%)					
$\frac{\Delta OD}{\Delta t}$	0.08	0.07	0.05	0.04	0.08	0.06
Protein % of dry weight	47	46	46	51	52	51

2.3. Effect of unsaturated fatty acid supplementation on the growth rate of brewer's yeast

The effect of linseed oil was investigated at 0.5% maltose concentration and 750 l h^{-1} l^{-1} aeration intensity in synthetic and malt germ media. When frozen yeast was used as inoculum the addition of unsaturated fatty acid in form of linseed oil the lag period shortened to only 2 h, in case of synthetic medium and only 1 h in malt germ medium.

The addition of oil caused a decrease in maximum growth rate in comparison to non- supplemented synthetic medium and the protein content of cell biomass was lower. Linseed oil supplementation at a level of 0.5% resulted in increase in growth rate and decrease in protein content when yeast was propagated in malt germ medium. This higher growth rate could be a result of co-utilization of the added oil. The lower oil concentration caused a decrease in the maximum growth rate (Table 3).

Table 3

Effect of linseed oil on the generation time $\left(\frac{\Delta OD}{\Delta t}\right)$ *of brewer's yeast, propagated in column fermentor at 0.5% maltose concentration and 750 l* $h^{-1}l^{-1}$ *aeration level*

	Synthetic medium				Malt germ medium		
	Oil concentration (%)						
	Control (0)	0.1	0.25	0.5	Control (0)	0.1	0.5
Growth rate $\left(\frac{\Delta OD}{\Delta t}\right)$	0.7	0.05	0.05	0.04	0.07	0.055	0.09
Protein % of dry weight	47	43	41	38	51	52	40
Length of lag period	3	2	—	2	3	1	1

2.4. Effect on inoculum preaeration on the length of lag period

When brewer's yeast suspension stored at −18 °C for weeks was used as inoculum and preaerated for 4 h the lag phase decreased by 2 h and more in synthetic medium, aerated at 750 l h^{-1} l^{-1}. The growth rates were not influenced by the treatment they show dependence on maltose concentration (Table 4, Fig. 4.)

In case of fresh brewer's yeast suspension preaeration proved to be especially effective so even in the first hour of preparation on synthetic medium an increase in yeast concentration could be detected.

Table 4

Effect of 4 h preaeration and maltose level (0.5–2.0%) on the growth rate of brewer's yeast propagated in column fermentor

Maltose (%)	Growth rate $\left(\frac{\Delta OD}{\Delta t}\right)$
0.5	0.070
1.0	0.065
2.0	0.050

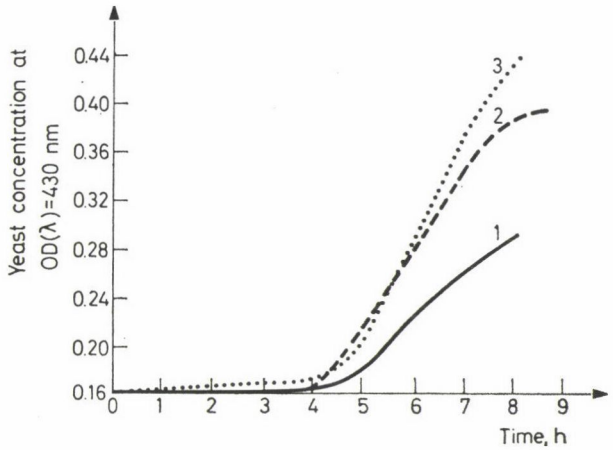

Fig. 4. Effect of maltose concentration on yeast growth of preaerated, frozen stored brewer's yeast (aeration: 750 $1h^{-1}1^{-1}$). 1: 0.5% maltose, tg $\alpha = 0.07$; 2: 0.1% maltose, tg $\alpha = 0.065$; 3: 2.0% maltose, tg $\alpha = 0.05$

3. Discussion

The productivity of brewer's yeast production can be increased by optimization of the maltose content and intensive aeration. If the yeast biomass is ment to be used as a protein source aeration level has to be chosen carefully.

Reduction of the long lag period is of great economical importance in brewer's yeast propagation. The lack of unsaturated fatty acids due to the almost anaerobic growth conditions during brewing are responsible for the long lag phase in case of inoculation with yeast suspension separated after the fermentation of malt worth.

Preaeration proved to be efficient, even 1 h pretreatment resulted in decreasing lag period. Prolonged preaeration affected not only the lag period but was benefitial for the growth rate too. As unsaturated fatty acids are essential for the cell wall synthesis and these compounds can be synthesized

only under aerobic conditions the positive effect of inoculum preaeration is understandable. Supplementation of medium with unsaturated fatty acids by adding linseed oil at various concentrations is especially effective in case of malt germ medium as the lag period was shortened to only 1 h. Higher amounts of linseed oil (0.5%) seemed to be cooxidized by brewer's yeast and resulted in increased growth rate. At the same time the protein content decreased significantly under fermentation conditions tested. This phenomenon is similar to that when yeast is propagated on fat as carbon and energy source, however it has not been mentioned until now that brewer's yeast is able to utilize plant oil.

Malt germ medium enriched with maltose is at least as good as synthetic medium for yeast propagation. Although no difference could be detected in the lag period between the two media without oil supplementation (in both cases 3 h) linseed oil at 0.1% proved to be more efficient in malt germ medium, so the unsaturated fatty acid content of the germ extracted into the medium might be of importance.

Frozen storage of brewer's yeast suspension caused an increase in the lag phase in comparison to fresh yeast suspension. As freezing and thawing causes the break down of yeast cell wall so the negative effect might be explained by the decrease of viable cells in the inoculum.

*

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CRITERIA OF ESSENTIALITY, BENEFICIALITY AND TOXICITY OF CHEMICAL ELEMENTS

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The author reviewed the criteria of essentiality, beneficiality and toxicity. It was demonstrated that the rigid classification of the different elements has not real value: the correlations between the nutritive elements need a very many-sided approach to have a real value. The scientific opinions on this topic were changed year to year as the consequence of newly discovered facts and correlations.

Keywords: essentiality, beneficiality, toxicity, element-interactions

It is worldwide accepted that the first form of life started about 3.5 thousand million years ago and the inorganic environment, i.e. the geosphere and the biosphere were and are now in very close correlation or interrelationship. In the first steps of biosynthesis the inorganic compounds of the Earth's crust, first of all — in soluble form — the different nutritive elements, including the trace elements, had a very active role.

About two decades ago — applying very sensitive new analytical methods — science demonstrated that living organisms contain (excepting the 6 noble gases) all the other 82 elements which are permanent components of the Earth. To avoid misunderstanding, we accentuate that it does not prove that all elements have an important role in the physiological processes of the biosphere, but it may mean, that it is not so easy to exclude any element from the possibility to have this type of role. This situation is to be seen in Fig. 1, which contains all the 65 trace elements that we know to have any role in life processes.

This periodical system does not contain the 6 noble gases and the six strong radioactive elements which may be excluded from the biosphere (Table 1). The 11 essential macroelements are also omitted. They are listed in Table 2.

1. Criteria of essentiality

The rigid rules of essentiality given by ARNON and STOUT (1939) were published more than 50 years ago. These rules are the following:

— The organism can neither grow nor complete its life-cycle in the absence of the element.

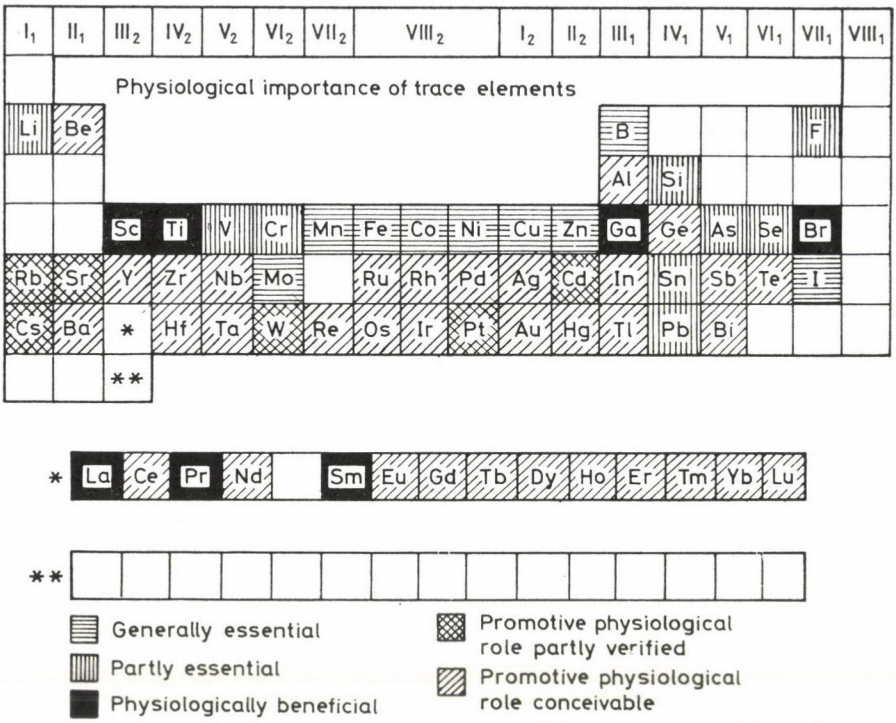

Fig. 1. Trace elements in the periodical system

— The element cannot be replaced completely by any other element.

— The given element has direct influence on the organism and is involved in its metabolism.

We would like to underline that these rules were accepted worldwide and in main relations are still valid now in spite of the fact that the basic viewpoints have changed in the last 2–3 decades and we have enough scientific

Table 1

Elements excluded from biosphere

Noble gases	Radioactive elements
Argon	Actinium
Helium	Polonium
Krypton	Protactinium
Neon	Radium
Radon	Thorium
Xenon	Uranium

Table 2

Essential macroelements

Symbol	Name	Symbol	Name
H	Hydrogen	O	Oxygen
C	Carbon	N	Nitrogen
P	Phosphorus	S	Sulphur
Cl	Chlorine	K	Potassium
Ca	Calcium	Na	Sodium
Mg	Magnesium		

experience to think over the whole problem. We should like to mention that BOWEN in his excellent book (1966) proposed some changes in these criteria. To point one: "the absence" should be changed as follows: "if the element is supplied at a sufficiently low concentration". To point three: the criteria should be extended: "To include cases where the given element is a pair of closely related elements".

In the late sixties and the seventies some new elements became accepted as partly essential to life: selenium, chromium, arsenic, tin, etc. We acknowledge one sentence of SCHWARZ (1970) as the basis of all future research work in the field of trace element science: to demonstrate that an element has essentiality, is a very hard task, but to exclude categorically from being essential, has no real basis. Out of 88 permanent elements, which are found in the Earth, there are — as we mentioned — only 12 elements which are unlikely to be essential to life: noble gases and the strong radioactive elements.

If we look over the most modern literature (SCHMIDT, 1989; GIBSON, 1990), nowadays we can calculate in living systems with 9 generally and 9 partially essential trace elements. The members of the first group are: boron, cobalt, copper, iodine, iron, manganese, molybdenum, nickel and zinc. In the second group we can calculate with the following elements: arsenic, chromium, fluorine, lead, lithium, selenium, silicon, tin and vanadium. These elements are listed in Table 3. It should be underlined that the classification of different elements is uncertain or it depends on personal opinions. For example the author never shared the widespread opinion in literature, that there exist only two groups of elements: essential and non-essential ones.

Possibly, it is surprising that we included arsenic and lead in the ,,partially essential" family of trace elements although according to worldwide accepted opinions these elements and also their compounds are highly toxic under common circumstances. In spite of this the mentioned essentiality is valid, but only under certain conditions and between very low narrow concentration limits.

Table 3

Essential trace elements

Generally	Partially
Boron	Arsenic
Cobalt	Chromium
Copper	Fluorine
Iodine	Lead
Iron	Lithium
Manganese	Selenium
Molybdenum	Silicon
Nickel	Tin
Zinc	Vanadium

2. Criteria of beneficiality

Now we should like to deal with the question on a broad scale: which are the elements and according to what criteria are they beneficial, i.e. have positive effects in different physiological processes. It means in other words: we traditionally accept the term "essentiality", but we believe that nowadays this is not so important and not sufficient to evaluate the physiological importance of different elements.

The second criterion of essentiality according to ARNON and STOUT (1939), that „the action of a trace element is specific and can not be substituted" is no longer valid. In the great monography of G. L. Eichorn we can find a short chapter dealing with the metal–metal replacements in the metal-activated enzymes (SCRUTTON, 1973). SHKOLNIK described also in his book (1984) different examples of trace elements that can substitute another one in plant life. KŐRÖS (1983) gave also a very demonstrative example in his book about the peptidase activity of carboxy-peptidase which originally contains zinc, but the application of cobalt may increase the activity by 60 per cent.

If we calculate the enormously high number of synergetic and antagonistic effects, i.e. when the given element promotes or hinders the physiological activity of others, the former picture is much complicated. To declare this or that element to be essential, correlates to many parameters, therefore we should concentrate our attention on the term of "beneficiality" in the near future.

Now we try to define the criteria of beneficiality. An element may be regarded as beneficial, if the following criteria exist:

— By application of the given element we can register promotive role in plant growth, in body weight gain of domesticated animals or in the health conditions of humans.

— After the application of the given element the activity of one or more enzymes with positive physiological effects, is increased.

— By application of the given element the plants, animals and human beings show better health conditions or better immunological responses to diseases.

— Applying the given element, the toxical effects of other elements are decreased or can be diminished ("antidotum" effect).

— By application of the given element the uptake and utilization of other essential elements can be increased.

— Applying the given element some well defined physiological parameters show an advantageous change, i.e. the cholesterol level in blood becomes lower, etc.

According to the mentioned criteria, which are the elements that can be regarded as members of the family of beneficial elements? Our first example is titanium, which was regarded earlier as an ineffective element in physiological processes.

Titanium is one of the most widespread elements of our planet. We can find it in form of TiO_2, some titanes and Ti-silicates, which are not soluble under common conditions and therefore not uptakeable by plants and thus this element is usually not a member of the food-chain.

Applying this element in the form of a water-soluble, pH-stable chelate compound (e.g. as titanium-ascorbate), the above mentioned situation can be totally changed. The titanium-ascorbate applied on the leaves of plants can cross the cell-membranes and this chelate can activate different enzymes in the cells. As a consequence of these activities we can register a higher and better carbohydrate and protein biosyntheses (PAIS, 1983; FEHÉR et al., 1985). The same compound in animal nutrition can help in the utilization of feeds: it results in a higher body weight gain without the decrease of quality of products (BOKORI et al., 1985; NAGY et al., 1986).

The beneficial role of titanium has some other aspects too: in different experiments we registered beneficial effects in the reproduction processes of animal-breeding. Moreover, if we apply the titanium-ascorbate as a part of the daily feed ration, the stock will be more healthy, the number of ill animals will be reduced, therefore we may suppose that it plays certain role in the animal immunology (PAIS et al., 1989).

As a second example we can mention gallium, which according to our experiences (KISS et al., 1985; SIMON et al., 1989) can improve the carbohydrate synthesis and also biosynthesis of chlorophyll in blue-green algae.

It was demonstrated that different rare elements can play beneficial role in different plant experiments (LIU, 1988). According to papers of HOROWITZ (1988), HOROWITZ and SCHLOSSBERG (1989) scandium can also be regarded as a beneficial element. Lastly ANKE and co-workers (1990) demonstrated new data on the deficiency of aluminium in goats.

3. Criteria of toxicity

Toxicity is also a very manysided problem. Some hundred years ago Paracelsus (Theophrastus Bombastus von Hohenheim, 1493—1541) has defined the materials which should be considered as toxic. On the basis of this old opinion we should like to give a very unusual definition: none of the materials (compounds) are toxic, or all materials (compounds) are toxic: the main point is the concentration, i.e. the quantity of the substance.

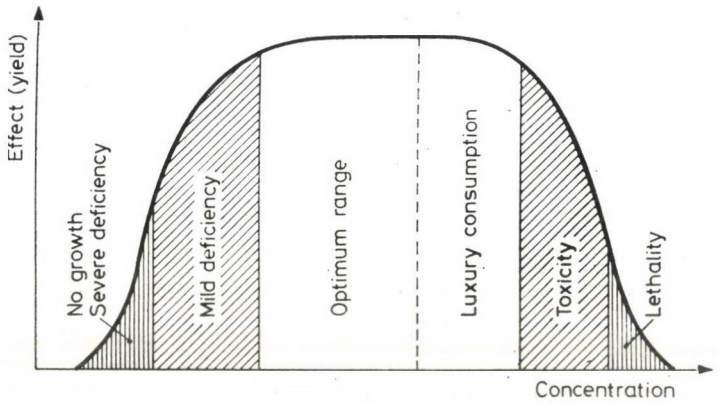

Fig. 2. Correlations between concentration and effect of elements

The most easily understandable example is sodium chloride (NaCl), that we use every day and it is not toxic in to 1–2% but at significantly higher proportion, it is definitely fatal for the human organism. One other, also well understandable example is the use of different medicaments, including some alkaloides. One or a few pills per day are very useful to help our organism fight against different diseases, but in ten or more times higher proportion they are lethal for human beings. This problem is demostrated in Fig. 2: all elements provoke deficiency at a very low concentration and on the other hand at a high level — including the generally essential elements! — toxicity symptoms. We should underline that these concentration ranges vary from element to element.

Finishing this part of this paper, we should show an other side of the term "toxicity" as well. As it was demonstrated in the last 10–12 years, there is no reason for speaking about the toxicity of this or that element, because we should like to draw attention to other elements which are present in the same system. To demonstrate element-interactions we should like to focus our attention on Fig. 3. The meaning of it is the following: some essential elements in an appropriate concentration can give a protection or may correct the toxicity of some dangerous elements, or if the supply of the essential ele-

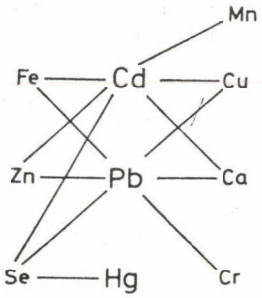

Fig. 3. Interactions of different elements

ments (e.g. zinc) is very low, the toxical effects (e.g. of cadmium) are more serious.

One other example: selenium is worldwide accepted as an essential element which provides protection against the development of certain types of cancer and circulation diseases, as well. On the other hand, we should like to mention that the "optimum-range" of selenium is very narrow: in greater concentrations it is highly toxic, or carcinogenic.

In the near future the term of beneficiality should have a much more important role in trace element science and the viewpoint of multidisciplinary approach should be the main "compass" in scientific efforts of the last decade of this century.

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EVALUATION OF ECONOMICAL PASTA PRODUCTS PREPARED FROM DURUM SEMOLINA/YELLOW CORN FLOUR/SOY FLOUR MIXTURES

I. MIXING PROPERTIES, CHEMICAL COMPOSITION AND COLOUR COMPONENTS

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Dry noodle pasta products were prepared from durum semolina (DS) and from several blends of DS with yellow corn flour (YCF), full fat soy flour (SF) and defatted soy flour (DSF). Mixing properties, chemical composition and colour components were examined. Increasing levels of YCF, SF or DSF in blends remarcably increased dough strength, stability during mixing, dough development time (DDT), mixograph curve area (CA) and water amount required for development. Increasing amounts of SF or DSF either alone or in combination with YCF significantly increased total pasta protein, fat, ash and brownness. DS/YCF blends produced pasta products of lower protein but exhibited the best colour scores.

Keywords: pasta quality, semolina/corn/soy blends, mixing properties, colour indices

High-quality pasta products are produced generally from durum semolina, and this explains the success of durum wheat in the world market. In some countries, such as France and Italy, there are laws prescribing that pasta products can be made from durum wheat only. However, for developing countries, such as Egypt, the production of pasta products from various native raw materials would be of economic significance. In addition, because of their high popularity, these products could widen the offer of habitual diets with improved nutritive value in these countries.

Whole corn flour has already been incorporated into pasta products as untreated and heat-treated with or without soy flour (MOLINA et al., 1975, 1976, 1982). WU and co-workers (1987) reported that acceptable high-protein and dietary fiber spaghetties can be prepared with up to 10% corn distiller's grains (a by-product of ethanolic corn fermentation). Corn gluten meal (a by-product of corn wet-milling industry) has been also incorporated, both alone and in combination with soy flour, into pasta products (BUCK et al., 1987).

Soy flour was used to supplement protein in macaroni products (PAULSEN, 1961, BREEN et al., 1977, HOPKINS, 1980) and to produce a quick-cooking pasta product (KINSLEY, 1965). Soy protein concentrates also have been used to produce high-protein pasta products by mixing with durum semolina (LAIGNELET et al., 1976).

Currently, in Egypt, although there is a considerable production of yellow corn and soybean, they are used mainly in animal feeding. Therefore, the purpose of this work was to introduce both yellow corn and soybean in the human nutrition in the form of acceptable, economical pasta products for the low income groups of people. Accordingly, several blends of DS with YCF, SF and DSF, both alone and in combination have been made, and their mixing properties, protein, ash, fat, carotenoid pigments and colour indices have been determined.

1. Materials and methods

1.1. Raw materials

DS, YCF, SF and DSF used in this study were commercial products available on the Hungarian market.

1.2. Preparation of blends

Several blends have been made as shown in Tables 1,3 and 4. Hundred percent DS was used as control.

1.3. Sample processing

Five kg of each blend were processed into pasta in the form of flat noodles in the Cereal Research Institute (Szeged, Hungary) with a laboratory-scale extruder at 50 °C die and extrusion temperature and at a vacuum of 733–800 kPa, respectively. Drying of noodles was carried out for 1 h at 40 °C and 90% relative humidity, followed by 14 h at 50 °C and constant 80% relative humidity, then the conditions were held at 25 °C and 66% relative humidity for the final 1.5 h.

1.4. Mixing properties

The ten-gram micro-mixograph technique of FINNEY and SHOGREN (1972) was used to compare the mixing properties of the doughs from several blends with those of DS dough, based upon the parameters: dough development time (DDT), maximum consistency (MC) and curve area (CA).

1.5. Chemical analyses

Moisture, protein ($N \times 5.75$), ash and fat were determined as described in A. A. C. C. methods (1983).

Carotenoid pigments were extracted overnight from 8 g of raw materials, both alone and mixtures as well as from processed pasta samples with

40 cm^3 of water-saturated *n*-butanol and light absorption of the clear filtrates was measured spectrophotometrically at 440 nm according to A. A. C. C. method (1968).

1.6. Colour indices

Colour was measured with the adapted disc-method of ALAUSE and FEILLET (1970) as previously described by TAHA & SÁGI (1987). Two indices are obtained: brown index (BI) and yellow index (YI). A well coloured pasta is characterized by a low BI and a high YI.

1.7. Statistical analyses

The data presented here are the averages of at least two parallel determinations.

Variance analysis, LSD at 5%, F type test and correlation coefficients were calculated with a Commodore 64 personal computer according to SVÁB (1981).

2. Results and discussion

2.1. Mixing properties

The mixogram of doughs prepared from pure DS and 14 raw mixtures obtained by the ten gram micro-mixograph technique are shown in Fig. 1, and their mixing parameters are summarized in Table 1.

As can be seen in Fig. 1, the mixograms of DS doughs change by the addition either of YCF or of SF. Dough strength and stability during mixing remarkably increased with increasing level of YCF or SF in the mixture. The addition of SF either in full-fat or in defatted form was more effective in this respect than the YCF.

The mixograph parameters reported in Table 1 revealed that blending YCF, SF or DSF with DS at any level delayed the developing time of doughs during mixing. This means that these mixtures need additional amounts of water to keep the developing time within suitable limits during processing. Additionally, normal mixograms cannot be obtained with some mixtures, such as DS/YCF/DSF 85/10/5 and 80/10/10 using comparable amount of water added (i.e. 5.5 cm^3 per 10 g of 14% m.b.), therefore, in order to obtain normal mixograms, the amount of water added should be increased (6.0 cm^3, Figs. 1, 13 and 15).

The mixograph CAs were larger in all mixtures compared to the CAs of the DS mixograms. This trend was more clear in the mixtures containing SF and DSF (mixtures No. 4–9, Table 1) than in those containing YCF (mixtures

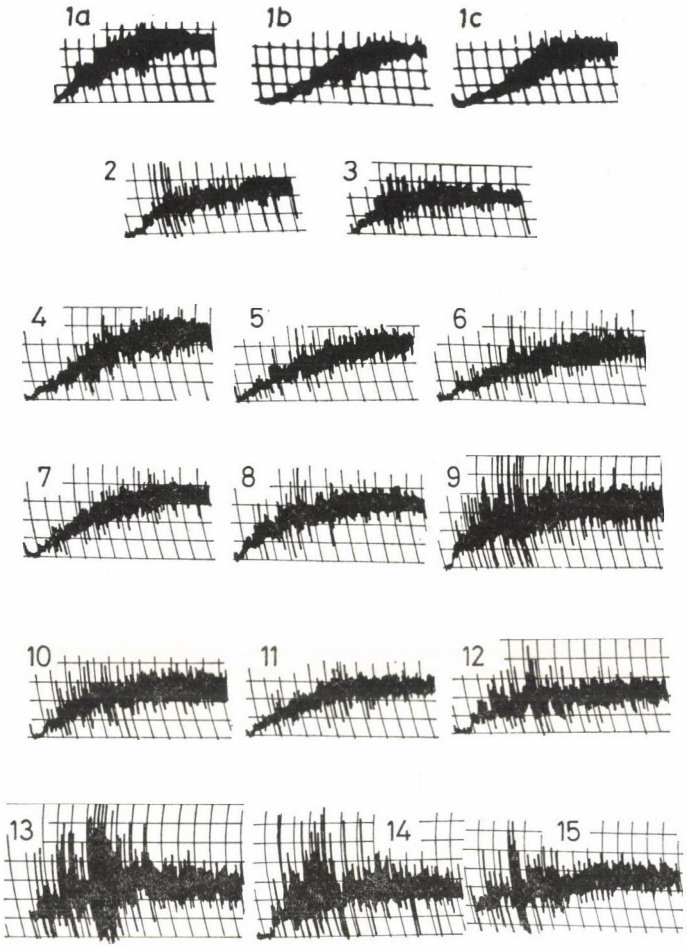

Fig. 1. Mixograms of durum semolina and durum semolina — yellow corn flour — soy flour mixture doughs (10 g samples of 14% moisture), mixing time 7 min, water absorption 5.5 cm³ (all mixograms) except 1-a (5.2 cm³), 1-c (5.6 cm³) 13 and 15 (6.0 cm³). 1-a, 1-b, 1-c: 100% durum semolina (control); 2: DS/YCF 85 : 15; 3: DS/YCF 75 : 25; 4: DS/SF 95 : 5; 5: DS/SF 90 : 10; 6: DS/SF 85 : 15; 7: DS/DSF 95 : 5; 8: DS/DSF 90 : 10; 9: DS/DSF 85 : 15; 10: DS/YCF/SF 85 : 7.5 : 7.5; 11: DS/YCF/SF 85 : 10 : 5; 12: DS/YCF/SF 80 : 10 : 10; 13: DS/YCF/DSF 85 : 7.5 : 7.5; 14: DS/YCF/DSF 85 : 10 : 5; 15: DS/YCF/DSF 85 : 10 : 10

No. 2, 3). This may be due to the higher protein content of SF mixtures (12.8–18%) compared to that of YCF mixtures (10.2–10.7%), since the mixograph CA showed a highly significant positive correlation with total protein content ($r = 0.70^{***}$, $n = 15$) as shown in Fig. 2.

The MC values as obvious in Table 1, seem to decrease as YCF increases in the mixtures while increase by blending of SF at 5% and DSF at 5, 10 and 15% levels. However, the other MC differences were statistically not significant.

Table 1

Mixing properties of durum semolina – yellow corn flours – soy flour mixture doughs

No.	Flour mixture				Mixograph values			Water absorption (cm³)
	DS (%)	YCF (%)	SF (%)	DSF (%)	DDT (min)	MC (Mu)	CA (cm²)	
1	100	—	—	— (cont.)	85	360	19.1	5.5
2	85	15	—	—	98	307	23.2	5.5
3	75	25	—	—	106	289	28.5	5.5
4	95	—	5	—	103	421	30.9	5.5
5	90	—	10	—	107	360	26.4	5.5
6	85	—	15	—	120	368	32.5	5.5
7	95	—	—	5	104	412	30.0	5.5
8	90	—	—	10	102	395	32.3	5.5
9	85	—	—	15	113	403	37.5	5.5
10	85	7.5	7.5	—	99	342	21.5	5.5
11	85	7.5	—	7.5	98	360	26.1	5.5
12	85	10	5	—	111	333	26.7	5.5
13	85	10	—	5	81	333	22.4	6.0
14	80	10	10	—	122	281	30.0	5.5
15	80	10	—	10	116	342	29.5	6.0
LSD 5%					9.6	29.4	1.47	
F test					9.7***	22.4***	108.1***	

*** Significant at P = 99.9% probability level

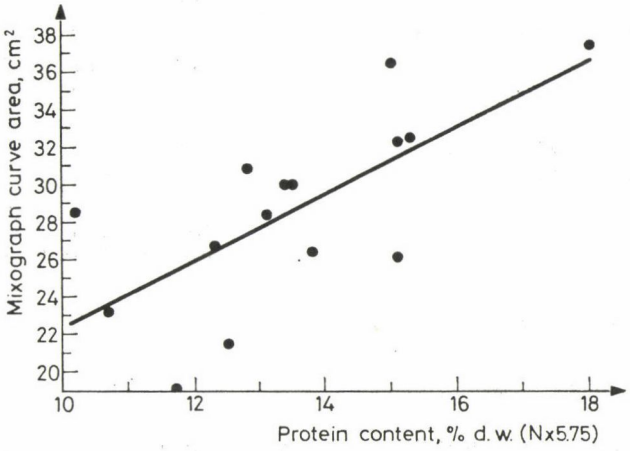

Fig. 2. Relationship between mixograph curve area and protein content of durum semolina and durum semolina — yellow corn flour — soy flour mixtures. ($y = 4.435 + 1.793$, $r = 0.703^{***}$, $r_{table(1\%)} = 0.6411$, $n = 15$)

Table 2

Chemical composition of pure durum semolina, yellow corn, full fat soy and defatted soy flours (d.w. basis)

Component	Flour			
	DS	YCF	SF	DSF
Moisture (%)	12.8	11.5	11.4	11.4
Protein (N×5.75, %)	12.7	5.6	38.5	49.7
Ash (%)	0.84	0.27	4.93	5.89
Fat (%)	1.49	4.23	18.2	2.23
Carotene (mg per kg)	6.59	41.6	29.3	20.7

Table 3

Chemical composition of pasta products prepared from durum semolina – yellow corn flour – soy flour mixtures

No.	Flour mixture				Content (%, on dry weight)			
	DS (%)	YCF (%)	SF (%)	DSF (%)	Moisture	Protein (N×5.75)	Fat	Ash
1	100	—	—	— (cont.)	10.1	11.7	0.19	0.71
2	85	15	—	—	10.0	10.7	0.38	0.66
3	75	25	—	—	9.3	10.2	1.00	0.61
4	95	—	5	—	9.3	12.8	0.90	0.84
5	90	—	10	—	9.1	13.8	2.15	1.09
6	85	—	15	—	8.8	15.3	3.38	1.34
7	95	—	—	5	9.3	13.4	0.33	0.98
8	90	—	—	10	9.4	15.1	0.53	1.20
9	85	—	—	15	9.1	18.0	1.06	1.51
10	85	7.5	7.5	—	9.0	12.5	1.46	0.96
11	85	7.5	—	7.5	8.9	15.1	0.28	0.99
12	85	10	5	–	9.2	12.3	0.74	0.80
13	85	10	—	5	9.4	13.1	0.35	0.90
14	80	10	10	—	9.1	13.5	1.40	1.06
15	80	10	—	10	9.2	15.0	0.62	1.13
LSD 5%					0.34	0.59	0.13	0.06
F test					6.50***	104.9***	381.4***	160.0***

*** Significant at P = 99.9% probability level

2.2. *Chemical composition*

Moisture, protein, fat and ash contents of pure DS, YCF, SF and DSF as well as of dry noodles processed from 100% DS and 14 mixtures are listed in Tables 2 and 3.

YCF contained about half the protein, and third the ash, but signifcantly more fat than semolina (Table 2). Accordingly, the noodles processed from DS supplemented with YCF was lower in total protein and ash, but significantly higher in fat than the controls (Table 3). In contrary, full fat SF had about 3 times the protein, 6 times the ash and 12 times the fat contents of pure DS. Over and above, the commercial DSF contained about 4 times the protein, 7 times the ash and 1.5 times the fat contents of the pure DS. Consequently, this resulted in significant increases of protein, ash and fat contents of the noodles processed from DS supplemented with SF or DSF either alone or in combination with YCF at all levels.

2.3. Colour components

As obvious from the data presented in Table 4, carotene content either of all crude mixtures or processed noodles was significantly higher than that of the control, except the 95% DS and 5% SF mixture. This is due to the higher carotene content of YCF, SF and DSF compared to that of DS (Table 2). Although the two crude mixtures of DS with YCF alone had the highest carotene content, about 50% of their carotene has been destroyed during processing by the oxidative enzymes of these materials. However, these mixtures gained the highest colour scores because of the lowest BI associated with relatively high YI, compared to those of other blends and of the DS control.

The products supplemented with DSF exhibited the lowest carotene processing loss (5.3–12.1%) and YI higher than in the control, especially at 15% level. However, these products had the highest BI values (33.8–35.9). Generally, high BI values are observed by blending semolina with soy flours. This observation can be explained through the established inferior effect of higher protein content of blends containing soy flours compared to those containing YCF of pure DS control (Table 3) on the BI. Since protein content was significantly and positively correlated with BI ($r = 0.62^{**}$, $n = 15$) and did not affect YI, as shown in Fig. 3. These results confirm those obtained by KOBREHEL and co-workers (1974), LAIGNELET and co-workers (1976) and TAHA and SÁGI (1987). Moreover, Fig. 4 shows that the ash content was in highly significant positive correlation with BI ($r = 0.66^{***}$, $n = 15$), in agreement with GILLIS (1963), KOBREHEL and co-workers (1974) and TAHA and SÁGI (1987). Consepuently, higher ash content of soy flour blends (Table 3) may also be involved in the increased brownness in their pasta products.

3. Conclusions

With the protein deficiency problem in the diet of low income groups, together with the economical situations in the developing countries, such as Egypt, incorporation of soy flours in pasta products is a good way to improve

Table 4

Carotene contents and colon scores of pasta products prepared from durum semolina – yellow corn flour – soy flour mixtures

No.	Flour mixture				Carotene content (mg per kg d.w.)		Carotene loss (%)	Colour score	
	DS (%)	YCF (%)	SF (%)	DSF (%)	crude mixture	processed pasta		yellow index	brown index
1	100	—	—	— (cont.)	6.6	3.8	43.0	27.8	29.6
2	85	15	—	—	11.4	5.5	51.4	28.0	22.9
3	75	25	—	—	15.0	7.4	51.0	31.7	21.2
4	95	—	5	—	7.4	5.5	25.7	29.6	30.0
5	90	—	10	—	8.6	6.9	19.1	30.5	31.9
6	85	—	15	—	9.1	7.8	14.3	26.6	28.6
7	95	—	—	5	6.4	6.0	5.3	28.2	33.8
8	90	—	—	10	8.4	7.4	12.1	28.1	35.7
9	85	—	—	15	8.7	8.2	5.5	28.9	35.9
10	85	7.5	7.5	—	10.1	8.0	20.4	29.6	31.0
11	85	7.5	—	7.5	10.1	7.9	21.5	27.7	24.4
12	85	10	5	—	9.8	8.1	17.5	27.7	25.6
13	85	10	—	5	9.7	8.1	16.8	31.8	26.3
14	80	10	10	—	11.1	8.8	20.3	29.9	25.3
15	80	10	—	10	11.4	8.8	22.7	28.1	28.7
LSD 5%					0.95	0.45	6.34	0.76	0.48
F test					48.9***	90.0***	47.9***	32.1***	803.1***

*** Significant at P = 99.9% probability level

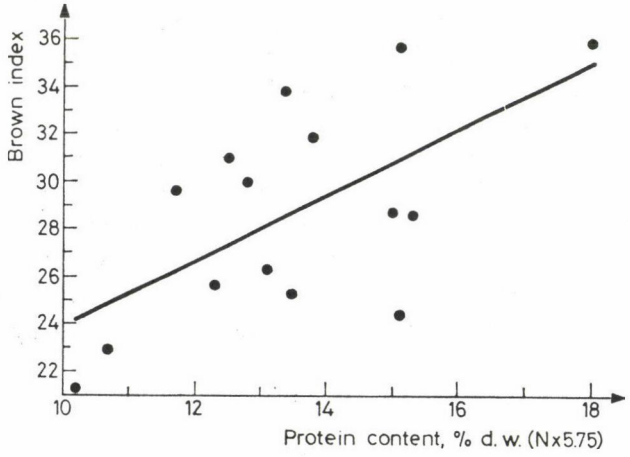

Fig. 3. Relationship between brown index and protein content of durum semolina and durum semolina — yellow corn flour — soy flour mixtures. ($y = 9.971 + 1.389x$, $r = 0.621^{**}$, $r_{table\ (2\%)} = 0.5923$, $n = 15$)

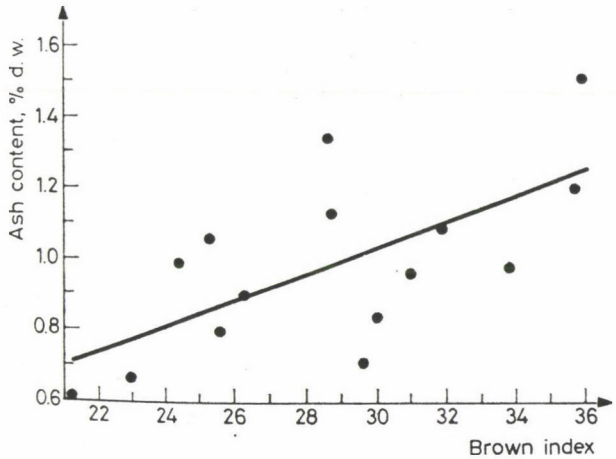

Fig. 4. Relationship between brown index and ash content of durum semolina and durum semolina — yellow corn flour — soy flour mixtures. ($y = -0.067 + 0.0366x$, $r = 0.657^{***}$, $r_{table\ (1\%)} = 0.6411$, $n = 15$)

both quality and quantity of proteins in pasta products. However, high levels of protein in pasta products supplemented with soy flours has an adverse effect on pasta colour by developing an unfavourable brownness. An improved yellow coloured pasta product can be processed by blending YCF with DS up to 25% without deleterious effects on protein quality, except the decrease of protein quantity.

Durum semolina–soy blends have a better nutritional value complementing each the other, since cereal proteins are deficient in lysine and tryptophan, whereas soy flour is deficient in S-amino acids. However, adding corn flour to durum semolina, lysine and tryptophan deficiency will be increased.

As far as processing is concerned, we did not find any important problems and all blends can be processed with the available extruders. However, higher amount of water is required to develop the dough during mixing and this must be determined before the extrusion. Therefore, drying of such pasta products will be more expensive with regard to the increased energy consumption.

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EVALUATION OF ECONOMICAL PASTA PRODUCTS PREPARED FROM DURUM SEMOLINA/YELLOW CORN FLOUR/SOY FLOUR MIXTURES

II. COOKING BEHAVIOUR, FIRMNESS AND ORGANOLEPTIC PROPERTIES

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Noodles were prepared from durum semolina (DS) blended with yellow corn flour (YCF), full fat soy flour (SF) and defatted soy flour (DSF) at several percentages both alone and in combinations. Cooking behaviour, firmness and organoleptic properties of the products were evaluated. All fortified noodles had a shorter cooking time, but their cooking losses were significantly greater. The addition of SF or DSF provided pasta products with a lower swelling, higher firmness and higher tolerance to overcooking than the 100% DS control. Addition of YCF had an opposite effect. Taste panel evaluation showed that noodles prepared with YCF up to 25% were acceptable for appearance, colour, odour and taste parameters. Noodles containing YCF + DSF in combinations were acceptable for appearance, odour, taste and mouthfeel parameters. Noodles made from 85% DS + 10% YCF + 5% DSF mixture were preferred over the other fortified noodles, and reached similar overall acceptability as the control (DS) noodles.

Keywords: pasta quality, semolina/corn/soy blends, cooking quality, organoleptic properties

Recently in Egypt more attention is given to increase yellow corn production using the new hybrids which are more homogenous, earlier maturing and higher yielding compared to their white counterparts. Soybean, one of the important native crops, is also widely produced in Egypt. However, utilization of these materials in the human nutrition is still very limited. Furthermore, the Egyptian pasta industry depends mainly either on the available bread flours for the production of economical, low quality products, or on the imported pure durum semolina for the production of expensive, high quality products.

Fortification of traditional pasta products, consumed throughout in Egypt by people of all ages and economic status, by adding corn flour or soy flour might provide an economical, new high-protein food in an acceptable form. But these products can be successfully introduced to the market only if they can reach the same quality as the similar standard commercial products. Therefore, in the present study, cooking behaviour, firmness and organoleptic properties of noodles processed from DS supplemented with YCF, SF and DSF,

both alone and in several combinations have been evaluated and compared to those made from pure DS as well as to two standard commercial pasta products.

1. Materials and methods

1.1. Sample processing

The available commercial products of pure DS, YCF, SF and DSF as well as standard 2-eggs and 4-eggs DS-pasta products were used in this study.

Several blends have been made as shown in Tables 1 and 2. Noodles were processed from each blend and dried as described previously in the first part of this study (TAHA et al., 1992).

1.2. Quality evaluation

1.2.1. Cooking test. Duplicates of 25 g dry noodles were cooked in 250 cm^3 boiling water for the optimum cooking time. Then, the samples were washed thoroughly with distilled water in a Buchner funnel, allowed to drain for 2 min, weighed to determine cooked weight, and transferred quantitatively into a graded tube containing known volume of petrol to determine cooked volume. The volume of the other uncooked sample (25 g) was determined similarly. The combined cooking and washing water was evaporated and the residue weighed to obtain the cooking loss. Cooking quality was characterized by the parameters: optimum cooking time (min), weight increase (%), volume increase (%) and cooking loss (%), according to the HUNGARIAN STANDARD (1958).

1.2.2. Firmness of cooked pasta. Ten discs (5 cm diameter) of dried pasta were prepared from each blend as well as from pure DS. Then, 5 discs were normally cooked (15 min) and the other 5 discs were over-cooked (30 min). The firmness of cooked discs was then determined using the aleurograph technique of SCOTTI and co-workers (1976).

1.2.3. Organoleptic properties. For the sensory evaluation, noodles processed from the several mixtures of DS with YCF, SF and DSF have been evaluated by 10 panel members (4 Egyptians and 6 Hungarians) compared to noodles processed from pure DS as well as to the two standard commercial pasta products. The samples were subjected to testing as dried and after cooking for 15 min. The following parameters were determined: appearance, colour, surface case, odour, taste, mouthfeel and fragmentation as described in the HUNGARIAN STANDARD (1958).

1.3. Statistical analysis

The statistical calculations were made with a Commodore 64 personal computer, according to SVÁB (1981).

Table 1

Cooking behaviour of noodles processed from durum semolina and durum semolina–yellow corn flour–soy flour mixtures

Noodles components				Optimum cooking time	Swelling		Cooking loss
DS (%)	YCF (%)	SF (%)	DSF (%)	(min)	Weight increase (%)	Volume increase (%)	(%)
100	—	—	— (cont.)	14.8	209.2	288.9	6.7
85	15	—	—	7.0	218.4	298.0	11.8
75	25	—	—	7.0	228.8	311.1	13.9
95	—	5	—	7.0	200.8	282.9	9.2
90	—	10	—	7.8	189.6	265.0	11.1
85	—	15	—	7.0	167.6	233.3	11.6
95	—	—	5	7.0	203.2	288.9	10.8
90	—	—	10	6.8	192.0	277.8	11.6
85	—	—	15	7.0	177.2	245.0	14.8
85	7.5	7.5	—	7.0	202.4	288.9	10.8
85	7.5	—	7.5	7.0	208.8	288.9	10.5
85	10	5	—	7.0	204.0	279.8	10.8
85	10	—	10	6.8	210.8	287.8	9.5
80	10	10	—	7.0	200.0	277.8	10.8
80	10	—	10	8.0	196.8	277.8	10.4
LSD 5%				0.85	8.49	10.23	0.49
F test				51.2***	29.3***	33.3***	135.1***

*** Significant at P = 99.9% probability level

2. Results and discussion

2.1. Cooking quality

2.1.1. Cooking behaviour of the noodles. The obtained results for cooking behaviour of dry noodles processed from pure DS as well as from the several blends of following composition: DS/YCF, DS/SF, DS/DSF, DS/YCF/SF and DS/YCF/DSF are given in Table 1.

It is clear that fortified noodles at all levels have a shorter cooking time than the 100% DS noodles. The optimum cooking time was reduced from 14.8 min for 100% DS noodles to 6.8–8.0 min for the blends. A similar reduction in cooking time was reported for soy blends (SCHARSCHMIDT & AUBLE, 1971; D'ALESSANDRO & D'ALESSANDRO, 1973) and for pea flour or pea protein concentrate blends (NIELSEN et al., 1980).

Swelling values, i. e. weight and volume increase of cooked noodles caused by cooking water absorption as percentages based upon the uncooked noodle

weight and volume (Table 1) were generally higher for the noodles containing only YCF compared to the 100% DS noodles. These increases were statistically significant except for the cooked volume increase at the level of 15% YCF added. On the contrary, the noodles containing SF or DSF showed lower swelling values than the control noodles. Cooked weight and cooked volume decreased with increasing level of supplementation. However, these decreases were not significant at the lowest addition level (i.e. 5%). These results confirm the preliminary statements made by PAULSEN (1961) on macaroni containing soy flour and the findings of LAIGNELET and co-workers (1976), who stated that addition of soy proteins lowers the swelling index in the cooked pasta. BUCK and co-workers (1987) also showed that the soy-containing pasta had lower cooked weight than the control or that containing only corn gluten meal.

The data presented in Table 1 show, however, no significant differences between swelling values of the noodles containing YCF and SF or DSF in combinations and of those made from 100% DS, except the blends of DS/ /YCF/SF (80 : 10 : 10) and DS/YCF/DSF (80 : 10 : 10), respectively, which exhibited a significantly lower weight increase.

All samples containing YCF, SF and/or DSF both alone and in combinations showed a significantly greater cooking loss, than the control samples. Cooking loss is undesirable, and according tu WU and co-workers (1987) it should not exceed 8% of dry weight. Among blends, cooking loss ranged between 9.2% for noodles containing 5% SF and 14.8% for noodles containing 15% DSF, whereas it was 6.7% for 100% DS (control). With increasing level of addition the cooking loss also increased. YCF and DSF pasta products were especially vulnerable to cooking, probably because the pregelatinization increased starch solubility. A similar high cooking loss was reported for soy-protein-blends (LAIGNELET et al., 1976) and soy flour blends (BUCK et al., 1987). Also WU and co-workers (1987) stated that the cooking loss increased as corn distiller's dried grains fraction content increased in spaghetti.

2.1.2. Firmness of cooked pasta. Firmness values (bar) of pasta after normal cooking for 15 min and overcooking for 30 min as determined by the aleurograph technique are listed in Table 2.

As obvious from the data presented, firmness values for pasta with added YCF were significantly lower than for the control at both 15 and 30 min cooking times. Firmness scores decreased as YCF additive percentages increased, in agreement with the results obtained for spaghetti with corn distiller's dried grain additives (WU et al., 1987) and for pasta containing corn gluten meal (BUCK et al., 1987). On the other hand, addition of SF or DSF provided cooked pasta with higher firmness compared to the control. DSF had the more prominent effect in this respect. With increased level of addition firmness values also increased (especially at 15 min cooking time). These results confirm the previous findings of PAULSEN (1961), LAIGNELET and co-workers (1976)

Table 2

Firmness of noodles processed from durum semolina and durum semolina–yellow corn flour–soy flour mixtures

Noodle components				Firmness (aleurograph values, in bar)			Firmness loss upon overcooking (%)
DS (%)	YCF (%)	SF (%)	DSF (%)	Cooking time (min) 15	Cooking time (min) 30	Total	
100	—	—	— (cont.)	60.9	25.0	85.9	58.9
85	15	—	—	54.8	21.4	76.2	61.0
75	25	—	—	47.3	16.2	63.5	65.8
95	—	5	—	59.8	30.3	90.1	49.3
90	—	10	—	64.3	31.8	96.1	50.5
85	—	15	—	75.6	34.8	110.4	53.7
95	—	—	5	70.1	36.9	107.0	47.3
90	—	—	10	74.1	35.3	109.4	52.4
85	—	—	15	94.2	37.3	131.5	60.2
85	7.5	7.5	—	61.4	23.0	84.4	62.5
85	7.5	—	7.5	65.0	32.0	97.0	50.8
85	10	15	—	49.8	19.3	69.1	61.2
85	10	—	5	53.8	17.8	71.6	66.9
80	10	10	—	56.2	20.8	77.0	62.7
80	10	—	10	59.7	19.8	79.5	66.6
LSD 5%				3.58	3.57	4.99	5.91
F test				87.9***	35.9***	112.9***	10.3***

*** Significant at P = 99.9% probability level

and BUCK and co-workers (1987). Likewise, at 15 min cooking time, pasta containing YCF/SF or YCF/DSF in combinations showed decreases in firmness values with increasing YCF level and with decreasing SF or DSF levels, and vice versa.

Values of firmness loss by overcooking as an index for the tolerance of pasta to overcooking revealed that 25% YCF additive resulted in less tolerance to overcooking compared to the control because its higher firmness loss (Table 2). Although the firmness of cooked pasta was generally increased as SF or DSF percentages increased, the tolerance to overcooking was decreased as SF or DSF increased. However, the values of firmness loss by overcooking for all samples containing SF or DSF remained significantly lower than in the control, except those with the highest level (15%) which were statistically not different from the control.

Table 3

Organoleptic quality parameters of noodles processed from durum semolina and durum semolina–yellow corn flour–soy flour mixtures

Sample description				Appearence	Colour	Surface case	Odour	Taste	Mouthfeel	Fragmentation	Total	Overall acceptability
				10[a]	10[a]	15[a]	5[a]	20[a]	30[a]	10[a]	100[a]	(%)
Noodles processed from:												
DS (%)	YCF (%)	SF (%)	DSF (%)									
85	15	—	—	7.8	8.5	11.4	4.6	16.5	20.3	7.2	76.3	83.6
75	25	—	—	8.2	9.1	12.6	4.1	16.6	24.2	8.7	83.5	91.6
95	—	5	—	8.2	7.2	11.8	4.3	17.1	23.6	9.0	81.0	88.8
90	—	10	—	7.0	7.0	11.8	3.9	16.3	24.3	8.1	79.8	87.4
85	—	15	—	7.3	7.3	10.6	4.0	14.9	20.8	8.1	73.2	80.1
95	—	—	5	6.9	6.7	10.9	4.1	16.2	20.9	7.8	73.5	80.5
90	—	—	10	7.1	7.0	11.5	3.8	14.9	23.3	6.5	76.4	83.7
85	—	—	15	6.7	4.8	8.6	3.2	11.8	18.9	6.3	58.3	63.6
85	7.5	7.5	—	7.8	7.3	11.6	4.5	16.0	24.1	8.8	78.0	85.6
85	7.5	—	7.5	7.8	7.8	12.5	4.3	16.0	25.5	9.2	82.9	90.8
85	10	5	—	6.7	7.4	10.9	4.2	15.2	23.7	8.4	74.5	81.6
85	10	—	5	8.6	8.5	13.0	4.5	16.2	27.1	9.5	87.4	95.8
80	10	10	—	8.1	7.5	12.1	4.5	17.0	25.3	9.1	82.1	89.8
80	10	—	10	7.8	6.8	12.4	4.3	16.1	25.0	9.0	81.4	89.0
100	—	—	— (cont. 1)	9.3	8.9	13.9	4.5	19.2	27.8	9.9	93.5	
Commercial product:												100.0
Two eggs 100% DS			(cont. 2)	8.8	9.1	14.4	3.0	17.3	25.8	10.0	88.0	
Four eggs 100% DS			(cont. 3)	9.9	9.6	14.1	3.6	17.3	26.9	10.0	91.7	
LSD 5%				2.06	1.15	1.52	0.61	2.13	2.76	0.88	6.62	7.20
F test				1.5 NS	8.3***	6.6***	4.4***	4.0***	6.4***	9.9***	12.1***	10.6***

*** Significant at P = 99.9% probability level
NS: Not significant
[a]: Maximum score

2.2. Sensory evaluation

Sensory evaluation data of noodles processed from 100% DS and from those containing 2 eggs and 4 eggs, respectively, (3 controls), as well as data of the fortified processed noodles were statistically analyzed. Means of the parameters: appearance, colour, surface case, odour, taste, mouthfeel, fragmentation and overall acceptability are shown in Table 3.

Panel members gave the best scores for the controls (without significant differences between them) for all sensory parameters except the odour test. Several panel members commented that the commercial pasta with 2 or 4 eggs had an objectionable odour. Noodles containing only YCF was generally preferred by the panelists over those containing only SF or DSF, because the relatively higher scores given to the YCF-noodles for appearance, colour, odour and taste parameters compared to those given to the SF or DSF-noodles. Moreover, the results of sensory evaluation indicated that the addition of YCF, SF and DSF to fortify pasta products in combination is preferred over to the addition of each alone. This is clear from the relatively higher scores given to the noodles prepared from combined flours for appearance, odour, taste, mouthfeel and fragmentation parameters compared to those given to the other noodle groups. Among all fortified noodles, the sample processed from 85% DS, 10% YCF, 5% DSF mixture had the highest total sensory evaluation score (87.4) and reached 95.8% of the overall acceptability of the controls mean. In other words, this product did not differ significantly from the control noodles.

3. Conclusions

In the present study, incorporation of some native raw materials such as corn (YCF) and soy flours (SF, DSF) in pasta products was not intended to replace regular, durum semolina-made (DS) pasta products, but to provide an economical, nutritious and high-protein staple food for low-income people and developing areas. However, noodles fortified with YCF/SF or YCF/DSF can also have desirable organoleptic properties and reach the same or nearly the same overall acceptability as DS and the commercial standard controls, as indicated by taste panel evaluation. Additions of YCF, SF and DSF at all levels reduced cooking time, but increased cooking loss. Cooked weight and cooked volume were increased as YCF additives increased, but decreased as SF or DSF additives increased. On the other hand, firmness of cooked pasta was decreased as YCF-additives increased, but increased as SF or DSF additives increased. Based upon these experimental data, especially noodles produced from a blend of 85% DS, 10% YCF and 5% DSF can be recommended as a new, advantageous pasta product, resulting in a better nutritive value and in saving durum wheat for other food purposes.

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OCCURRENCE AND EFFECT OF INDIGENOUS AND EVENTUAL MICROBIAL ENZYMES IN LACTIC ACID FERMENTED VEGETABLES[a]

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1. Introduction

Long since historical time food has been preserved by fermentation. Inspite of the introduction of modern preservation methods, lactic acid fermented vegetables still enjoy a great popularity, mainly because of their nutritional and sensory qualities.

There are some characteristic features in the production of fermented vegetables which will be pointed out below using cucumbers as an example. In the production of lactic acid fermented cucumbers the raw material is put into a brine without previous heating. Through the effect of salt and oxygen deficiency the cucumber tissues gradually die. At the same time the semipermeability of the cell membranes is lost, whereby soluble cell components diffuse into the brine and serve as food substrate for the microorganisms. Under such specific conditions of the brine the lactic acid bacteria succeed in overcoming the accompanying microorganisms and lactic acid as the main metabolic product is formed. Under favourable conditions (for example moderate salt in the brine, use of starter cultures) it takes at least 3 days until the critical pH value of 4.1 or less — desired for microbiological reasons — is reached.

Besides the typical taste, for the consumer a cracky texture is the most important quality criterion for fermented vegetables. Figure 1 shows the factors which can influence the texture, whereby the enzymes (especially the pectinolytic enzymes) are particularly important. Because there is no heating step before the fermentation, the indigenous plant enzymes in the fermenting materials are still present during the very first phase. After the destruction of the cell membranes they easily get to their active sites and under favourable

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conditions they can easily cause softening. The environmental conditions act in different manner on the single enzymes or enzyme systems: some enzymes are strongly inhibited by salt, others are activated, and in the acid pH-region many enzymes are irreversibly inactivated. Besides indigenous enzymes also enzymes produced by microorganisms can be responsible for the undesired soft products.

2. Indigenous enzymes in cucumber

2.1. Isolation of the enzymes

For the isolation and characterization of the enzymes and their quantitative determination in raw and fermenting cucumbers an enzyme extract was obtained by means of extraction and purification steps (e.g. filtration and

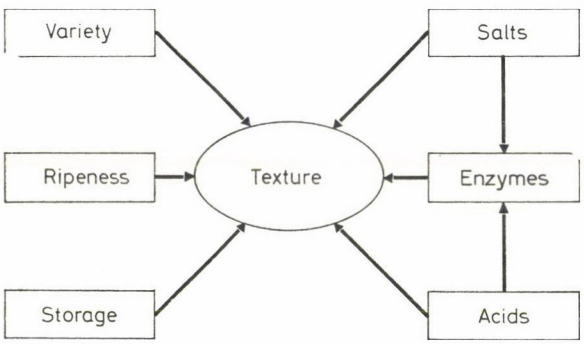

Fig. 1. Factors influencing the texture of fermented vegetables

centrifugation) and concentration by ultrafiltration (Fig. 2). For all the enzyme investigations an enzyme extract obtained by this method was used. This procedure was quick and reproducible.

2.2. Characterization of the enzymes

2.2.1. Pectinesterase (PE). Pectinesterase (Pectinmethylesterase, EC 3.1.11) catalyses the hydrolysis of highly esterified pectin to less esterified pectin and methanol. This deesterification is the prerequisite for the splitting of the glycosidic bonds of the pectin by the cucumber polygalacturonases. On the other hand calcium ion bridges are built between the carboxyl groups set free whereby a net of calcium pectate is produced. This leads to more firmness of the cell wall and a better resistance to enzymatic attack than with unnetted pectin.

Figure 3 shows, that the pH-optimum for the pectinesterase lies in a solution with 0.05 mol l^{-1} NaCl at pH 9.8 and what is not to be seen with

100 g raw material	cooled (4 °C) or deep-frozen
↓	
200 cm³ extraction solution **100 mg saponin**	0.75 mol l^{-1} NaCl solution, cooled
↓	
crashing	1 min in mixer
↓	
homogenization	1 min with ultra turrax
↓	
adjusting the pH value	pH 6.0 with 5 mol l^{-1} and 0.5 mol l^{-1} NaOH *(when fermented cucumbers are used)*
↓	
extraction	stirring at 4 °C for 2 h
↓	
filtration by suction	Büchner funnel with the help of nylon cloth
↓	
centrifugation of the extract	4 °C, 25 min, 19.000 g
↓	
concentration by ultrafiltration	Minitan tangential flow filtration, cooling with ice pressure: 15 psi ≙ 1 bar
↓	
dialysis	2 times for at least 4 h at 4 °C in 2 dm³ dialysis solution *(0.01 mol l^{-1} acetate buffer pH 5.6, 0.1 mol l^{-1} NaCl, 0.001 mol l^{-1} Na-azide)*
↓	
centrifugation	4 °C, 20 min, 30 000 g

Fig. 2. Scheme for extraction of the enzymes from cucumbers

0.15 mol l^{-1} NaCl the pH optimum lies at pH 7.8. Under acidic conditions, to have optimum activity, a certain ionic strength is necessary (Figs. 4 and 5). At pH 4.0 in the absence of salt the pectinesterase is completely inactive. Remarkable is the high temperature stability; OMRAN and co-workers (1991) report a z'-value of 14.9 °C. The influence of temperature on the deesterification of pectin is shown in Fig. 6 (MEURER et al., 1990). According to CHUN and co-

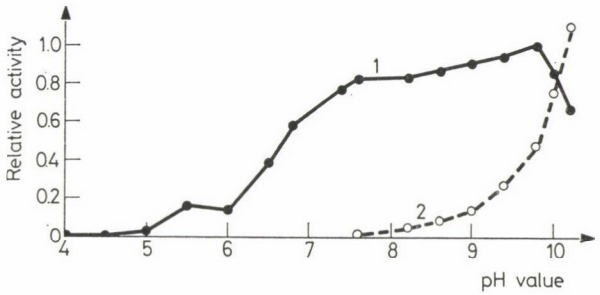

Fig. 3. pH optimum of pectinesterase. (NaCl concentration of the substrate: 0.05 mol l^{-1}; cucumber variety unknown). 1: pH optimum; 2: blank values

workers (1986) pectinesterase is totally inactivated when it is heated for about 20 min at 75 °C.

2.2.2. Exo-polygalacturonase (PG). Exo-PG (EC 3.2.1.15) hydrolyzes α-1,4-glycosidic bonds of polygalacturonic acid from the non-reducing end (PRESSEY & AVANTS, 1975). The velocity and the extent of the hydrolysis increases with the degree of deesterification of the pectin. It is supposed that two carboxyl groups beside each other are necessary for the action of the polygalacturonase. Exo-PG in cucumber has a pH optimum of about 5.0 (Fig. 7) and it is less heat stable than the pectinesterase. It is strongly inhibited by salt (Fig. 8) as well as by $CaCl_2$ concentrations of above 0.4 mmol l^{-1} (0.006%) (Fig. 9). On the other hand a minimum of calcium ions is necessary for the action of polygalacturonase. By adding EDTA or citrate and therefore complexing the calcium, the activity is completely lost (PRESSEY & AVANTS, 1975).

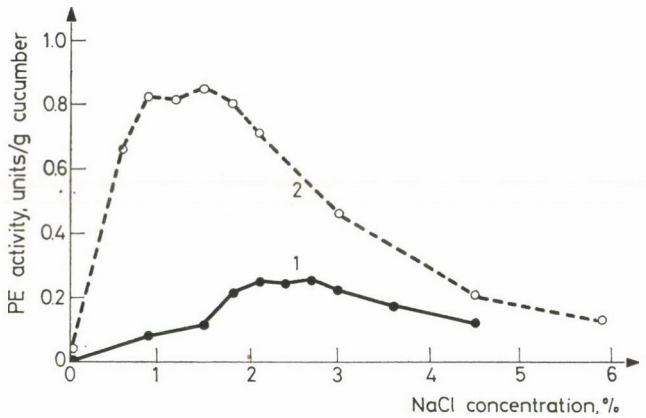

Fig. 4. Pectinesterase activity dependent on the NaCl concentration. 1: pH 4.0; 2: pH 6.0

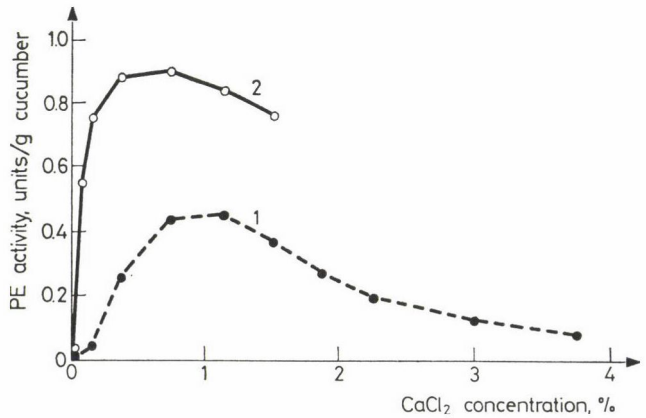

Fig. 5. Pectinesterase activity dependent on the $CaCl_2$ concentration. 1: pH 4.0; 2: pH 6.0

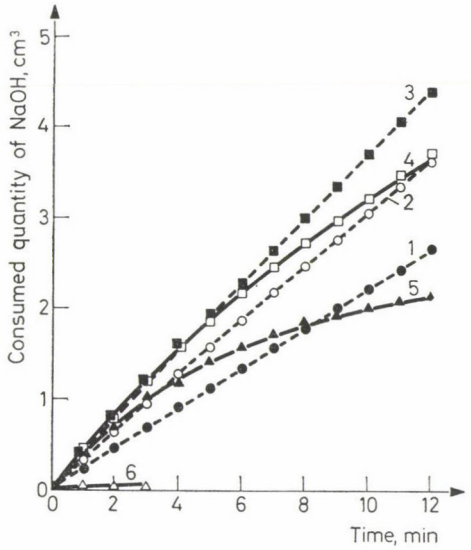

Fig. 6. Influence of the temperature on the enzymatically catalyzed deesterification of pectin (enzyme extract from cucumbers of unknown variety). 1: 40 °C; 2: 50 °C; 3: 60 °C; 4: 70 °C; 5: 75 °C; 6: 80 °C

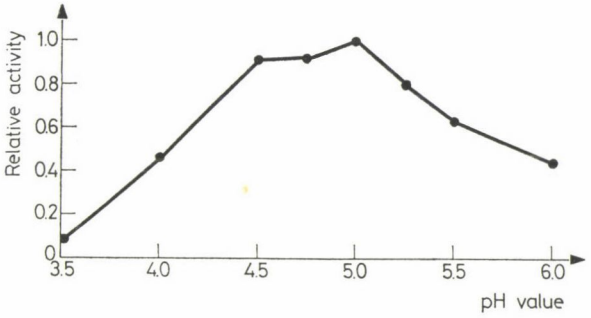

Fig. 7. Influence of the pH value on the activity of exo-polygalacturonase

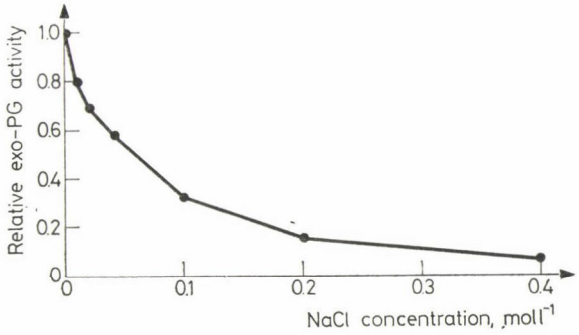

Fig. 8. Influence of the NaCl concentration of the reaction mixture on the activity of the exo-polygalacturonase (from cucumbers of the "Othello" variety)

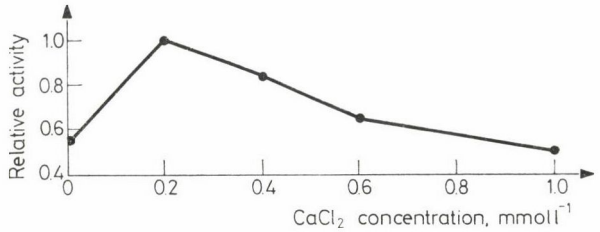

Fig. 9. Influence of the $CaCl_2$ concentration on the activity of the exo-polygalacturonase (from cucumbers of the "Othello" variety)

2.2.3. Endo-polygalacturonase. With the help of an enzyme extract from cucumber it is possible to split citrus pectin (pectin C, degree of esterification 70–75%). Supposedly this is caused after the effect of PE by endo-polygalacturonase which can also break down polygalacturonic acid at lower velocity. In comparison to exo-polygalacturonase it is not inhibited by citrate. A NaCl content of 2.5% in the enzyme solution leads to complete loss of activity. At the beginning of the fermentation the activity of endo-PG increases sharply before it is inactivated like C_x-cellulase due to the decreasing pH value (Fig. 10). The observed increase in activity could be due to enzyme production in the changed anaerobic metabolism of the cucumbers after packing in the jars for fermentation. Production of the enzyme by the fermentation microorganisms is unlikely because in the heated and then fermented cucumbers there was no endo-PG activity. The characteristics of this enzyme are similar to those of endo-PG found in mature cucumber by McFeeters and co-workers (1980). Its pH optimum is 5.6 and its optimum ionic strength is at $\mu = 0.2$.

2.2.4. Endo-β-1,4-glucanase (C_x-cellulase). The endo-β-1,4-glucanase (C_x-cellulase, EC 3.2.1.4) splits cellulose hydrolytically at the non-crystallized region. Since the elemental fibrils of the primary cell wall of higher plants are composed of cellulose, the C_x-cellulase can cause softening of fermented

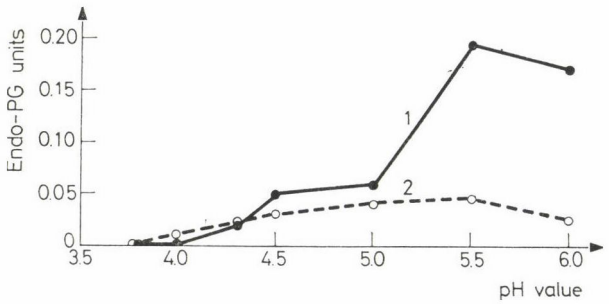

Fig.10. Influence of the pH value on the activity of the endo-polygalacturonase (from cucumbers of the "Christine" variety, fermented for 2 days). 1: pectin C; 2: Na-polygalacturonate

vegetables. Added C_x-cellulase of microbial origin clearly causes softening in cucumber when stored for more than 4 months and this can not be prevented by the addition of calcium (BUESCHER & HUDSON, 1984). In Fig. 11 the dependence of the indigenous endo-β-1,4-glucanase activity on the pH value can be seen. Experiments of the authors show that already after a few days of fermentation when the pH value goes below 4.8 the cucumber C_x-cellulase is

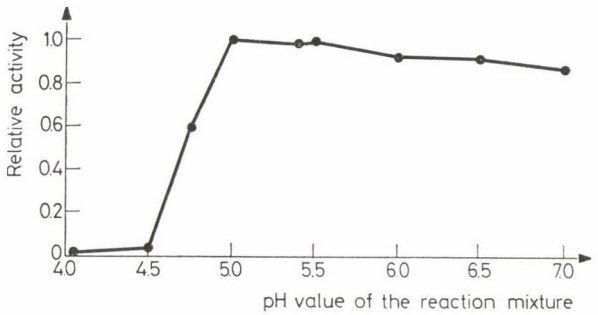

Fig. 11. Dependence of the endo-β-1,4-glucanase activity on the pH value of the reaction mixture (enzyme extract from cucumbers of the "Christine" variety)

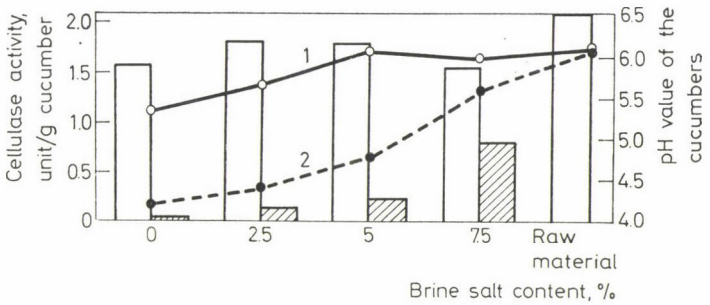

Fig. 12. Inactivation of cucumbers C_x-cellulase due to decreasing pH: □: 2 days of fermentation; ///: 5 days of fermentation; 1: pH value on the 2nd day: 2: pH value on the 5th day

irreversibly inactivated (Fig. 12). In contrast to polygalacturonase it is only weakly inhibited by high concentration of NaCl.

2.2.5. Glucomannan splitting enzymes. It was established that glucomannan is strongly attacked by cucumber enzyme extract. Glucomannan is a linear polysaccharide that is made up of randomly arranged 1,4-substituted β-D-glucopyranosyl and 1,4-substituted β-D-mannopyranosyl units. Depending on origin the ratio of D-glucose to D-mannose ranges from 1 : 1 to 1 : 4. The mechanism of the action of the enzyme is not yet known. Since only a few reducing end groups are set free in relation to the decrease in viscosity of the substrate solution it must be an endo-enzyme. The pH optimum lies at 5.5

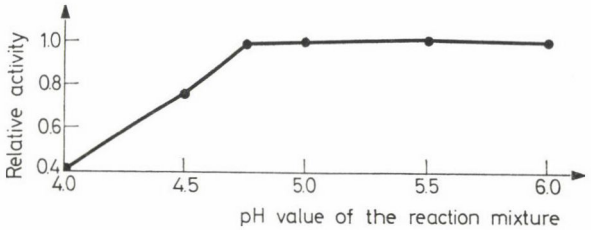

Fig. 13. Influence of the pH value on the activity of glucomannan splitting enzymes (cucumber variety "Othello", incubation time 15 min)

(Fig. 13). Its activity is reduced to half by a salt concentration of 0.25 mol l^{-1} (1.5%). In contrast to endo-β-1,4-glucanase the glucomannan splitting activity is still effective at pH 4.0 but in the same way it is during fermentationt otally — even though at a slower rate — destroyed.

2.2.6. Some specific exo-glycosidases. Table 1 gives an overview of some exo-glycosidases which were found in cucumber. Of these enzymes β-glucopyranosidase is clearly inhibited by NaCl. Apart from α- and β- galactopyranosidase all the exo-enzymes investigated were totally inactivated after 4 or 7 days of fermentation, depending on the salt content of the brine. Since the substrates for these enzymes are found in the cucumber cell wall, softening caused by these enzymes can not be ignored.

2.2.7. Proteases. The primary cell wall of higher plants in the model according to Keegstra and co-workers (Fig. 14) contains protein according to TALMADGE and co-workers (1973) in an amount of 5–10%. Because of its high content of glycosides it is also known as glycoprotein. With protease 0.3% of the cell wall proteins can be set free from untreated cell wall material and after treatment with endo-PG this fraction rises to 2% according to ALBERSHEIM (1976). Proteases destroy enzymes as well, and in this way they can play a part in the maintenance of the texture. In fresh cucumber a weak protease activity is present which decreases during the fermentation. In a solution with pH 6.0 the activity was twice as high as at pH 4.0.

Table 1

Exo-glycosidases of cucumber (variety Othello)

Enzyme	pH optimum
α-D-Galactopyranosidase	5.5
β-D-Galactopyranosidase	4.8
β-D-Glucopyranosidase	4.8
α-D-Mannopyranosidase	4.5
β-D-Xylopyranosidase	4.5
α-L-Arabinofuranosidase	5.0

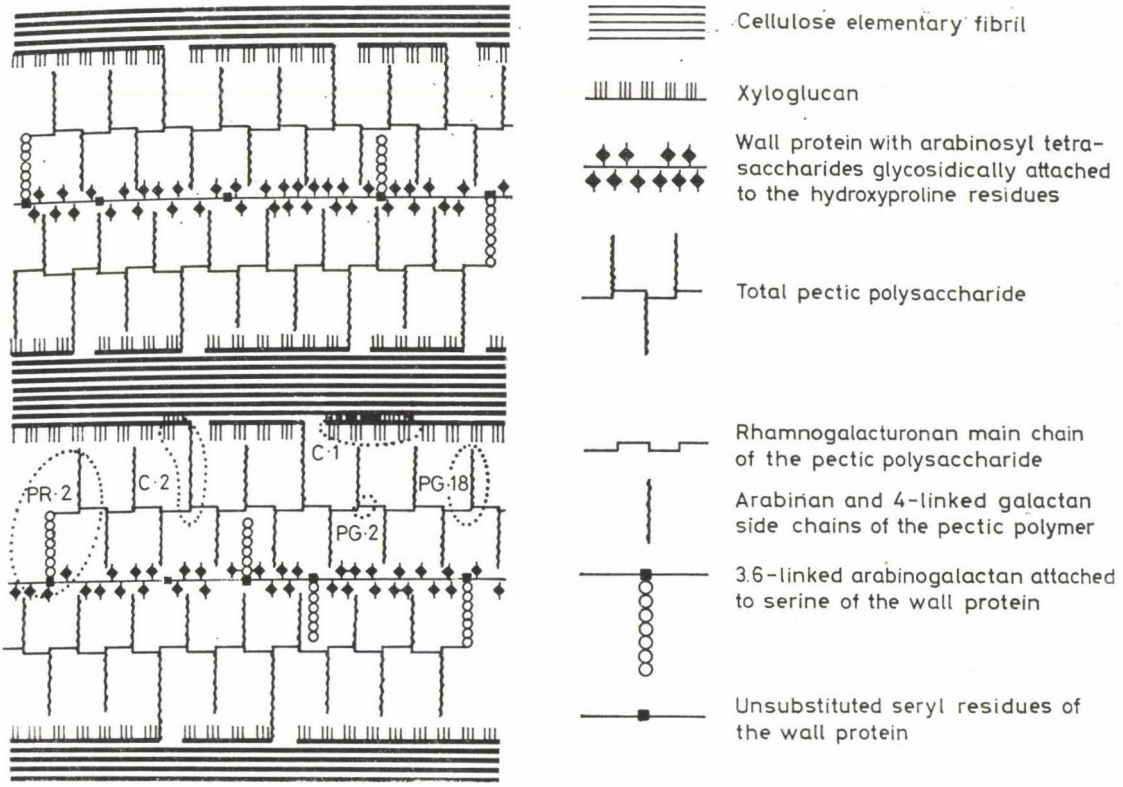

Fig. 14. Model for the cell wall of young plant tissue according to Keegstra and co-workers (1973)

2.3. Further enzymes and enzyme inhibitors

Cell wall bound peroxidase and some other enzymes catalyse the production of hydrogen peroxide (H_2O_2). According to MILLER (1986) H_2O_2 can oxidize some cell wall polysaccharides including pectin and cellulose. This oxidation leads to splitting of glycosidic bonds and therefore depolymerization of the polysaccharides. An incubation of cucumber cell wall material with a physiologically possible H_2O_2 concentration of 1 mmol l^{-1} sets free arabinose, galactose and xylose (MILLER, 1986). This cell wall bound peroxidase was not included in our investigations.

Indigenous plant enzyme inhibitors as found by BOCK and co-workers (1966; 1975) were not investigated in our experiments.

3. Distribution of the enzymes in the cucumber

It is important to know the distribution of the structure relevant enzymes within the cucumber in order to have a better understanding of the pro-

Table 2

Distribution of some enzyme activities within the 3 main morphological regions in cross section of the cucumber

(each relative to the lowest activity, referring to the cucumber fresh weight)

Enzyme	Exocarp	Mesocarp	Endocarp
Pectinesterase	12	2	1
exo-Polygalacturonase	1.5	1	2
C_x-Cellulase	4	2.5	1

Table 3

Remaining enzyme activities in preheated cucumbers
(percent of the activity in untreated cucumbers)

	Temperature (°C)		
	55	65	79
Pectinesterase	52	42	1.5
exo-Polygalacturonase	29	16	1
C_x-Cellulase	25	10	0

cesses which lead to softening. Table 2 shows the ratio of the most important enzyme activities based upon the fresh weight of the cucumbers within the 3 main morphological regions in cross section of these fruits. Striking is that the pectinesterase content in the skin is 12 times higher compared to the endocarp, while in the endocarp a little bit more polygalacturonase is present than in the other sections. The pectinesterase is supposedly bound to the cell wall and is set free by the influence of ions at the beginning of the fermentation after destruction of the cell membranes. The enzyme can then diffuse into all regions of the cucumber and into the brine. The part of pectinesterase increases with the salt content of the brine which was found in the brine after 14 days of fermentation (Fig. 15)

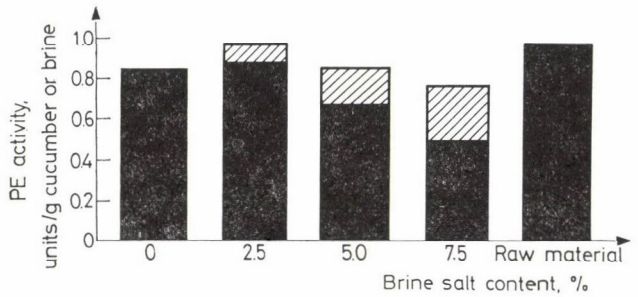

Fig. 15. Distribution of pectinesterase in fermented cucumber and in the brine. Fermentation time: 14 days. ▪: PE in cucumber; ////: PE in brine

Likewise the distribution of the enzymes in the longitudinal section of the cucumber is of interest because the cracky texture in fresh as well as in fermented cucumbers decreases from the stem end to the blossom end. The activities of PE, exo-PG and C_x-cellulase amount lowest in the stem third of the cucumber and in the blossom third the highest that means: the lowest "density" of texture relevant enzymes led to the best crackyness and vice versa.

4. Fermentation experiments with different salt concentrations in the brine

Salts have not only a direct influence on the texture (e.g. reinforcement of tissues by Ca^{2+} ions) but also their effect on the activity of the polysaccharide degrading enzymes is obvious. In order to be able to investigate the influence of salt (NaCl) on the crackyness of fermented cucumbers 8 fermentations were carried out with different salt contents in the brine. In addition to salt, in some of the fermentations $CaCl_2$ was added. To prevent the growth of molds and yeasts the brine contained 0.26% potassium sorbate; further a starter culture *(Lactobacillus plantarum*, DSM 20205) was used for inoculation at a concentration of 10^7 cells per cm^3 brine. After 15 days the cucumbers in their fermentation containers (2550 cm^3 jars) were pasteurized. Figure 16 shows the degree of crackyness of the cucumbers from the 8 fermentation experiments before and after pasteurization.

As the ratio of cucumbers to brine was 1 : 1 in the jars, the concentration of the salt in the cucumbers was reduced to about 50% due to diffusion. The results show that with a 1% salt content in the brine at the beginning, the crackyness was the lowest and with an 8% salt content it was the highest. An addition of 0.2% calcium chloride led to noticeable improvement of the

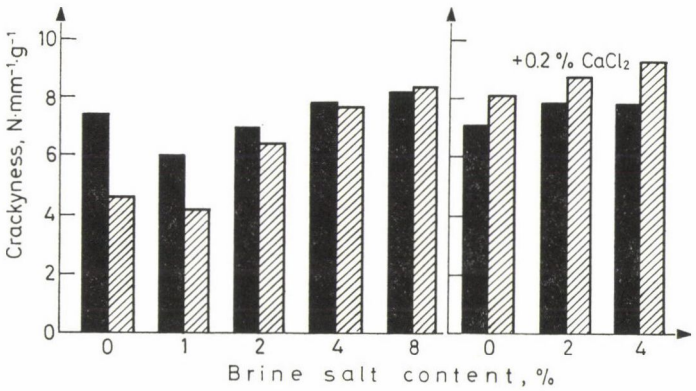

Fig. 16. Crackyness of cucumbers fermented in brine with different salt contents. Fermentation time: 15 days. ■: not pasteurized; ////: pasteurized

crackyness. Remarkably the samples with 0% and 1% NaCl were associated with the highest loss in texture during the heating step. During fermentation, a change in the degree of esterification of the pectin in the cucumbers could be observed. The fresh fruits' pectin showed a degree of esterification (DE) of 44% while the cucumbers fermented without salt had a DE of 39% after 4 days of fermentation. The cucumbers fermented in a 4% salt brine had a DE of less than 20%. The DE value of the pectin in the other samples fluctuated between the two values.

The observed effects can eventually be approximately explained by the following processes:

— The polygalacturonase activity of cucumbers was inhibited by high NaCl content (Fig. 8) as well as by the concentrations of $CaCl_2$ which were used.

— With the same high salt content the pectinesterase is especially active, a fact which leads to high deesterification of the pectin in the cucumbers. Without the addition of NaCl or $CaCl_2$ the PE is almost inactive in that acidic pH range and therefore the pectin in the corresponding fermentation sample is hardly deesterified.

— Calcium contributes to the maintenance of the texture through formation of stable insoluble calcium pectates or calcium pectate chelates and indeed the stronger the deesterification of the pectin the better is the effect.

— The sample without NaCl had better texture values than the one with 1% NaCl because the less deesterified pectin can poorly be broken down by PG. Presumably also less PG is able to get to the active site on the cell membrane because the brine from the cucumbers fermented without salt had the lowest PG activity (Fig. 17). Exo- and endo-PG have approximately the same activity under the same conditions. Beside that at very low ionic strength (under $\mu = 0.027$) the endo-PG of the cucumber should be inactive.

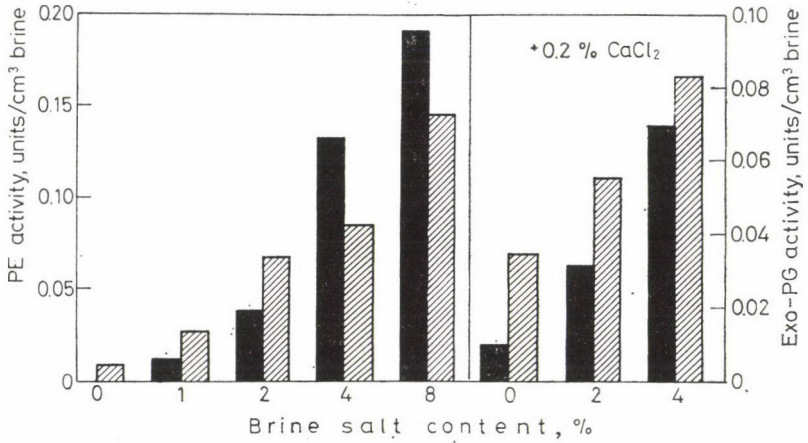

Fig. 17. Enzyme activities in the brines after 4 days of fermentation. ■: PE; ////: exo-PG

5. Cucumber fermentation after heating the raw material

Since pectinesterase is more heat stable than exo- and endopolygalacturonase, it should be possible, through heating the cucumbers before the fermentation to inactivate the PG sooner than the PE and perhaps to avoid softening in this way. By heating at temperatures higher than 80 °C the enzymes mentioned are completely destroyed, so that further conclusions could be drawn concerning the effect of these enzymes during fermentation.

Cucumbers of the Othello variety were heated at three different temperatures (until the core temperature of 55 °C, 65 °C and 79 °C was reached). The fruits from these three heat treatments as well as an untreated control group

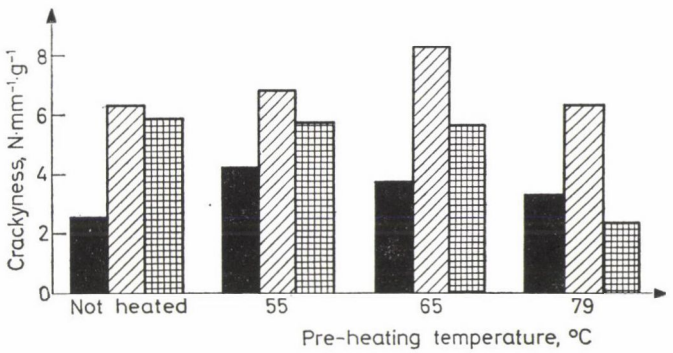

Fig. 18. Influence of the pre-heating temperature on the crackyness of cucumbers. ■: 1% NaCl; ▨: 1% NaCl + 0.2% $CaCl_2$; ▦: 6% NaCl, 14 days of fermentation, pasteurized

were fermented with three different salt contents (1% NaCl, 1% NaCl + + 0.2% $CaCl_2$, 6% NaCl) using *L. plantarum* as starter culture. After 14 days all the samples were pasteurized. Figure 18 shows the crackyness values of these cucumbers after an additional storage time of 4 months; Table 3 contains the data on the remaining enzyme activities which were found in the heated cucumbers. In contrast to the control samples a clear improvement of the texture of the fermented products was obtained by heating to 55 °C or 65 °C before fermentation and using low salt concentrations. On the one hand this might be due to the strong reduction of the PG activity. On the other hand in the samples, treated by heating and by addition of calcium ions, a faster diffusion of these calcium ions through the cell membranes could take place, because the membranes had lost their semipermeability by heat denaturation. Through the remaining activity of PE the pectin in the cell wall region could be deesterified; with the calcium ions chelates could be formed which are mainly responsible for the resulting reinforcement of the cell walls. Al-

ready in 1976 KEIJBETS and co-workers showed as you can see in Fig. 19 a model for the mechanism of this effect at the example of potato tissue. The samples with 6% NaCl showed no texture difference among them probably because the PG at such salt concentration is almost completely inhibited.

Especially noticeable was the extreme softening of the cucumbers heated at 79 °C and then fermented in 6% salt brine. Most probably difference from the others arise from the absence of the effect of the pectinesterase. It is

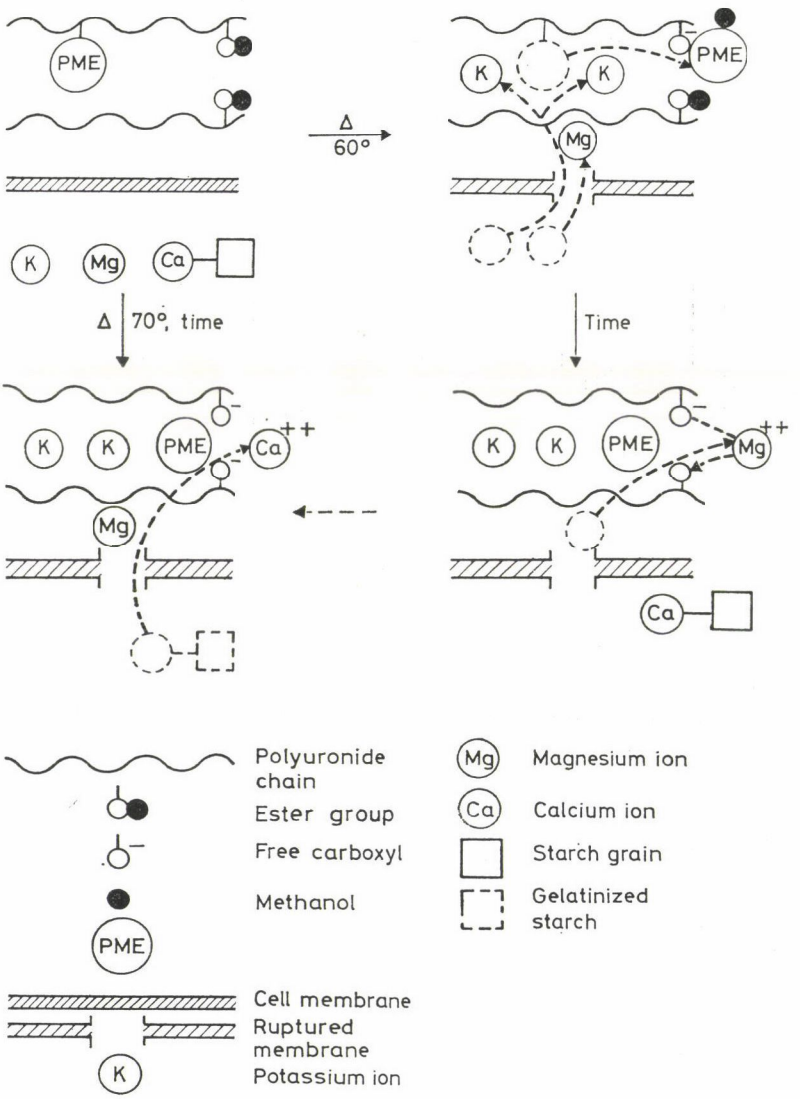

Fig. 19. Model for the reinforcement effect on the cell wall of preheated potato tissue caused by PE-catalyzed deesterified pectin and calcium ions

possible that with an increase in the ionic strength either the chemical hydrolysis of the pectin or the solubilization of the pectin chain out of the cell wall increases, while this increase diminishes with decreasing degree of esterification of the pectin.

Also without prior enzyme action calcium has a stabilizing effect, because the unchanged pectin of the cucumbers is only about 45% esterified (GESSLER, 1991) and therefore there are enough free carboxyl groups available for the netting by the calcium ions.

Finally it is to be mentioned that in the fermentation of Chinese cabbage a noticeable improvement of the texture could be reached by pre-heating and addition of $CaCl_2$ (BAEK et al., 1989).

6. Simulation of the fermentation process

In order to be able to investigate the effect of the added salts and acids on the texture independently 0.05% sodium azide (NaN_3) was added to the brine to prevent the growth of microorganisms. Figure 20 shows the crackyness values of the cucumbers "model fermented" under the described conditions before and after pasteurization. Particularly noticeable is the drastic loss of texture in the sample whose brine contained 2.4% citric acid. Probably the cause lies in the removal of calcium by the complexing effect of the citric acid, because the cucumber polygalacturonases are not active anymore under the adjusted pH value of 3.15. The cucumbers in lactic acid (pH = 3.4) remained cracky even after pasteurization. The altogether disadvantageous effect of acids on the texture can be related to removal of positive charged bivalent cations since the addition of calcium clearly contributes to the maintenance of the texture. In addition the pectinesterase activity is strongly inhibited by the influence of the acids and as a result of the lower deesterification less calcium bridges can be built.

7. Enzymes produced by microorganisms

Many bacteria, yeasts and molds can produce enzymes which break down the polysaccharides and can partly lead to a disasterous effect on the texture of the fermented product. In fermentation without the use of starter cultures and NaCl in the brine, the cucumber cell walls were so strongly attacked already after 14 days that the skin could easily be pealed off and the whole fruit could be effortlessly squeezed between the fingers.

There was a clear development of pectin-C-splitting enzyme activities which were present in the brine still after 3 weeks and which were not inactiv-

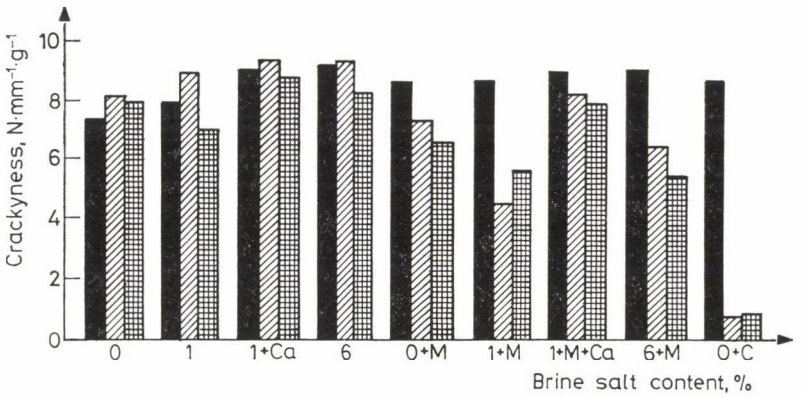

Fig. 20. Crackyness of "model fermented" cucumbers with different additives in the brine (C = 2.4% citric acid; M = 1.96% lactic acid; Ca = 0.2% $CaCl_2$). Prevention of microorganisms' growth by addition of 0.05% NaN_3. ■: 15 days, not pasteurized; ▨: 17 days, pasteurized; ▦: 122 days, pasteurized

ated by the effect of acids. Supposedly the salt-sensitive microorganisms which could grow in the weak acid medium in the first stage of fermentation were responsible for the synthesis of the enzymes mentioned.

It is generally known that molds and yeasts which grow on the surface of the brine can contribute to softening of cucumbers but up to now this has hardly been precisely investigated.

Recently it has been revealed that lactic acid bacteria including *Lactobacillus plantarum* can produce polygalacturonase (SAKELLARIS et al., 1988; JUVEN et al., 1985). In practical operation it must be cleared up, if and under what conditions starter cultures can lead to softening of fermented vegetables.

8. Closing remarks

Many processes which go on at the same time during the fermentation of vegetables influence each other and in total contribute to the quality of the product. Until today only a small section of these complex effects has been sufficiently studied, so that many questions can not or can only hypothetically be answered. Nevertheless today the texture of lactic acid fermented cucumbers can be improved when the knowledge about the consequences of the effect and the properties of the different enzymes can be put into practice. In principle this knowledge can also be of use in the fermentation of other vegetables which are fermented raw, however one has to take into account that the properties of the enzymes can differ depending on the vegetable type and — not infrequently — even on the variety.

9. Summary and outlook conclusions

Using cucumbers as an example it was demonstrated, that indigenous enzymes of plant raw materials essentially influence the achievement or failure of particular technological targets. In the case of lactic acid fermented cucumbers obtaining a cracky product is to be aimed at.

An essential step to achieve that is the thermal elimination of cell wall degrading enzymes (e.g. polygalacturonases) and the activation of pectinesterase after the cell membranes have lost their semipermeability and calcium ions can diffuse into the regions of cell walls which they reinforce.

In the meantime in our institute, time–temperature programmes are being worked out to obtain optimum firmness of several processed vegetables. Furthermore preliminary work has been started to obtain well-aimed technological effects by intelligent exploitation of special indigenous enzymes in plant raw materials which are to be processed. An example is the elimination of hydrocyanic acid from cassava which contains cyanogenic glycosides.

The author is convinced that the exploitation of indigenous enzymes for specific technological purposes will be an important field of work also in many other food technology institutes in the coming future.

The development of aroma substances from precursors and the preventive elimination of negative taste and coloured substances are examples for which the effect of indigenous enzymes mentioned above can be utilized.

*

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BOOK REVIEWS

Food preservatives

N. J. RUSSELL and G. W. GOULD (Eds.)

Blackie, Glasgow and London, 1991, 290 pages

This book written by world authorities on the subject is a major contribution to the literature of food preservation. It covers broader areas of the complex subject of antimicrobial food additives than that the title might suggest. The first two introductory chapters deal with a concise description of main characteristics of food-poisoning and food-spoilage micro-organisms, and factors affecting their growth and survival in major food preservation techniques. They are followed by individual chapters updating extensive information on: "Acidulants and low pH", "Organic acids and esters", "Sulphite", "Nitrite", "Solutes and low water activity", "Antibiotics — nisin", "Ethanol as a food preservative", "Modified atmosphere and vacuum packaging", "Phenols and chelators", and "Starter cultures". Besides effects on microorganisms and aspects of practical use, great emphasis is given in each of these chapters on mechanisms of action. A separate chapter summarizes present status and trends in legislation of preservatives, both at international (EEC, Codex Alimentarius) and national levels, in the latter with particular attention to the UK and USA. The final chapter ("Future prospects") addresses the growing interest in the potential for the improved, more deliberate use of natural antimicrobial systems and predictive mathematical modelling of microbial growth and survival in foods. Each chapter contains impressive selections of references covering the subjects until 1990. The book is highly recommended to food preservation professionals in industry and research, as well as to post-graduate students in food science and microbiology.

J. FARKAS

Enzymes in food processing

G. A. TUCKER and L. F. J. WOODS (Eds.)

Blackie, Glasgow and London, 1991, 304 pages

Use of enzymes in the food industry has been already a significant utilization of applied biochemistry before new biotechnological skills, genetic and protein engineering opened new perspectives for their more expanded use. With the advent of new biotechnologies, this book, which combines present day theoretical information with up-dating practical aspects of enzyme utilization in food industries, is a timely and most welcome guide and useful source of reference. Following the first two introductory chapters summarizing basic information on enzyme activity and general applications and limitation of enzymes in the food industry, new methods of encapsulating of enzymes, solvent engineering to alter enzyme specificity, development of catalytic antibodies are described in detail together with fundamentals of protein engineering. Individual chapters deal with specific applications of enzymes in milk and cheese production, the meat industry, the baking industry, the production of beverages and fruit juices, the starch and sugar industries, and the processing of fats and oils, respectively. The final chapter reviews various applications of enzymes as diagnostic tools and future trends and perspectives

for their use in food analysis and rapid detection and quantification of particular microorganisms. Extensive lists of references complete each chapter. The book provides a valuable resource and gives practical assistance to those who are interested in the challenging areas of current uses of enzymes and the potential of their increased uses in the future.

J. FARKAS

Natural food colorants

G. A. F. HENDRY and J. D. HOUGHTON (Eds.)

Blackie, Glasgow and London, 1991, 280 pages

The book gives a comprehensive overview on the natural pigments and food colorants available in the commercial trade. More than 400 natural pigments are reviewed giving a detailed overview on their structures, stability and natural presence. All those biochemical, chemical and technological characters of the listed pigments are reported which are indispensable for food science and technology. The book groups the colorants in 7 chapters, all the chapters are completed with detailed references. Altogether 967 references can be found giving a rich treasury of information on the respective field of science.

The first chapter entitled "Natural pigments in biology" introduces the major and at least the most widespread of the minor pigments occurring naturally in plants, fungi, lichens, animals and bacteria. It reports on the classification of the pigments on the basis of their chemical structure as well as their systematizing according to the colour and defines them in accordance with their place and role in the biological system.

The second chapter "Natural food colours" involves the issues of systematization, classification and authorization. A special stress is laid upon reviewing those food colorants being in everyday use at food production. So such food colorants as annatto, anthocyanins, beetroot, turmeric and carmine are discussed in details together with their application fields.

The third chapter deals with "Chlorophylls and chlorophyll-derivatives. In this part the chlorophylls and their derivatives are discussed as well as their structure, biosynthesis, natural occurrence, behaviour, stability and also the applicability in foods.

The fourth chapter entitled "Haems and billins" overviews those pigments which are the most visually apparent in nature, from blood-red to the azure blue of marine algae. Their nomenclature, structure, function, occurrence, biosynthesis, metabolism, physical properties, stability are discussed here as well as their uses in food industry.

The fifth chapter deals with the "Carotenoids". The carotenoids are natural colorants used widely. Due to their importance this chapter discusses all aspects of carotenoids, especially in relation to their presence or use in food. Topics discussed include the distribution of carotenoids, particularly in plants and animals, the natural functions and commercial uses and applications of carotenoids, the pathways and regulation of carotenoid biosynthesis, the absorption and transport of carotenoids and their metabolism, especially into Vitamin A. A separate part of the chapter is devoted to the natural and synthetic carotenoids as colourants and to the physicochemical determination method.

"Anthocyanins and betalains" is the title of the sixth chapter. In this part the chemistry and biochemistry of anthocyanins and betalains — their structure and distribution, biosynthesis, factors, influencing their stability, methods of extraction and analysis, and current and potential sources and uses — are reviewed. It is remarkable that this chapter itself contains 470 references.

The last (7th) chapter bears the title "Miscellaneous colorants". Those colorants are discussed here which are originated from various sources according to the literature though they are very important constituents of food colorants. Out of these the following items are discussed in details: Acylated β-ring substituted anthocyanins, annatto, saffron, gardenia pigments, cochineal and related pigments, turmeric, carthamin, monascus. This chapter is completed with 260 references.

The book can be used as a help in solving issues in research activity as well as in practical technology therefore may be recommended to all who are engaged in this field.

É. SZÁNTÓ-NÉMETH

Lebensmitteltechnologie. Entwicklungen und Aspekte

H. RUTLOFF (Ed.)

Akademie-Verlag, Berlin, 1991, 708 pages

This standard work written by 32 authors embraces in 14 chapters the whole field known today as "Food biotechnology". Moreover, it is completed by a subject index and a list of microorganisms as well as of abbreviations not generally known, or used in this book only.

The first chapter deals, after an introduction, briefly with all the subsequent chapters, giving at the same time the reasons why they have been included in this work. Chapter 2 (32 pp. by H. VOSS) is devoted to fermentation techniques and to a generally rather neglected field of biotechnological engineering, i.e. downstream processing of bioproducts including separation of cell mass. Chapter 3 (A. LEUCHTENBERGER) deals with genetic aspects of the improvement of microorganisms. Obtaining of microbial proteins and their processing to food are subjects of chapter 4 (G. KLAPPACH–E. LIPPERT–U. KRETZSCHMAR–H. BÖHM–O. PULZ) including all the groups of microorganisms used for this purpose. The most comprehensive chapters (a total of 273 pages) are devoted to enzymes. Chapter 5 treats soluble enzymes with special emphasis on starch modification (F. SCHIERBAUM–M. VORWEG), proteolytic including milk clotting enzymes (U. BEHNKE–A. TÄUFEL–W. KIRCHHÜBEL), pectin modifying (W. BOCK–G. DONGOWSKI) and cellulose/hemicellulose decomposing enzymes (W. F. HIRTE–G. SCHULZ). Chapter 7 deals with immobilised enzymes and cells as well as their most important production procedures and fields of application (R. BERGER–M. GOMOLL). Between those two large chapters, a smaller one (No. 6) is devoted to an often neglected field not well enough understood, i.e., enzyme inhibitors (A. TÄUFEL–J. HEMPEL). Chapters 8 to 10 deal, in the order as follows, with biotechnological aspects of amino acids (H. JESCHKEIT–C. GRIEHL), microbial polysaccharides (K. KRÜGER–L. FIEDLER) and organic acids used in foods (U. STOTTMEIER–K. HOPPE). Chapters 11 (H. BÖHM–H. HERMERSDÖRFER) and 12 (H. RUTTLOFF–M. ROTHE) on cell cultures and aroma and flavouring substances, respectively, are, to a certain extent, related. Therefore some unavoidable, however, slight overlapping can be observed in them. Finally, chapter 13 (P. LIETZ–M. TISCHLER–K. ZICKRICK–E. ACKERMANN) deals with selected traditional foods, including starter cultures for fermented milk and meat products, while chapter 14 (H.-J. LEWERENZ) is devoted to the extremely important field of aspects of nutritional toxicology of biotechnology.

The excellently compiled Tables and Figures enable the reader to get an easy insight in the individual fields and details of biotechnology. The references enlisted at the end of each chapter consist mainly of relevant primary literature of the past 15 years (up to 1987 !). The short historical surveys and trends of development at the beginning and the end, respectively, of each chapter illustrate the results already achieved and emphasize crucial topics for future R & D, respectively. The printing technique used contributes to an easy surveying of the individual fields. Summarizing the above said, the book can be considered as an encyclopedic standard manual of food biotechnology.

L. VÁMOS-VIGYÁZÓ

Plastic films. Technology and packaging applications

K. R. OSBORN and W. A. JENKINS

Technomic Publ. Co., Inc., Lancaster—Basel, 1992, 258 pages

This publication deals with a very actual topic: the main properties of plastic films, their production and their utilization for packaging. The book also discusses all those modifying processes with the help of which the polymer foils could be more suitable for packaging. Further, those main trends are reviewed to be awaited in the choice and the use of polymer foils in packaging, with special consideration of future requirements.

The book is divided into seven chapters, completed with glossary and index. The first chapter discusses the molecular structure of polymers and the physical, chemical,

mechanical features due to this structure. This chapter is ended with a bibliography. The second chapter deals with methods of the manufacture of plastic films, the technological processes with special consideration to the aspects of economy, also completed with bibliographical data. The third chapter outlines the modification technology of plastic films. These processes are discussed in their entirety being useful for the modification of polymer film properties in consideration of market requirements expressed by the users. This chapter is completed by bibliographical data, as well. The fourth chapter presents the requirements due to the packaging function of the material in a comprehensive mood. The fifth chapter gives account of the most important machines applied in the packaging techniques used for the packaging of dry powders, of granular and liquid goods, or even solid products in individual packages. A special part is reserved for the heat sealing equipment. This section has been completed by a bibliography, too. The sixth chapter deals with the problems of packaging on the different domains of application, putting a special emphazis on the processes of food packaging. Besides, the themes of commercial packaging, the packaging of health care articles, and of products in packages of great sizes, the protection of the loads with wrapping are discussed. This has been completed by a bibliographical summary, as well. The seventh chapter estimates the trends of the future and the possibilities deriving from demands, analyzing governmental, sociological, economical aspects and their technological consequences. In the appendix, the packaging industry has been reviewed, main properties of plastic films and resins are introduced quoting market prices, with a comprehensive character.

The book can be used effectively so by the manufacturers of packaging materials as by the professionals using them, but at the same time, it can be offered to all those who study — either theoretically or practically — the problems of packaging development. Last but not least, the book can be consulted and used effectively by students of highschools and universities who are interested in packaging techniques.

I. VARSÁNYI

Flour confectionery manufacture

C. A. STREET

Blackie and Son Ltd., Glasgow, 1991, 234 pages

Publishing this book supplies a long-felt want because no comprehensive study has been edited in this theme since the 70-ies.

After the informative introduction the author deals with the basic materials of flour confectionery manufacture including their qualitative kriteria.

Discussing the auxiliary materials besides the up-to-date theoretical considerations a stress is laid upon studying the natural and synthetic flavour- and colour-materials and upon the evaluation of the differences, as well.

The treatment of the physical and chemical changes during product manufacturing is carried out according to up-to-date viewpoints, in some details, however, it is much more profound than an average.

Producing processes of the basic products of flour confectionery is discussed in nine groups and even at discussing the coating materials of the flour confectionery besides the composition the theoretical relations are also dealt with.

Quality control and quality protection take also a significant part in the book in which not only the description of methods can be found but the way of practicing the control, as well.

The up-to-dateness of product development may be especially reasoned by the fact that not only descriptive receipts are given but the variation possibilities are often written in a mathematical formula theoretically supported. By this way a possibility is opened for a preliminary determination of the wholesomeness, the qualitative, the dietetic and economic criteria of the new products. Because of this chapter not only for the experts of flour confectionery but of the baking industry as a whole, too, this book may be of great interest.

In addition to this the book can be recommended to researchers in food science, to technologists as well as to professors and students engaged in this field.

L. SZALAI

The composition of foods

R. A. McCance and E. M. Widdowson

5th revised and extended edition. The Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food, Letchworth, U. K., 1991, 462 pages

The book deals with the composition data of foods. In the fifth edition of the UK food composition tables, at present 1188 foods are included. The tables are usable in nutrition, dietetics and catering. The tables give the major important nutrients of foods. In the first part of the book the General Instructions, being important in using the food tables, can be found. The tables include the foods, grouped according to their main characteristics, as cereals, milk, fat, oils, meat, fish, vegetables, nuts, sugars, fruits, beverages, confectionary, cheese. The tables contain the data for each food separately. After the number and name of food, the edible proportion, the energy value and the major nutrients as protein, fat, carbohydrate are given. The other part of the table contain the nitrogen, fatty acid totals, cholesterol, starch, total sugars and dietary fibre content as well as the inorganic elements — Na, K, Ca, Mg, P, Fe, Cu, Zn, Cl, Mn, Se, I — and the vitamin composition of the respective food — retinol, carotene, vitamin B1, B2, B6, B12, D, E, C, folate, panthotenate —. The data of the foods are explained by the General Instructions. E.g. the Factors for converting total grams of nitrogen in foods to protein, the Conversion of carbohydrate weights to monosaccharide equivalents, the Relationship between the dietary fibre fractions, the Energy conversion factors, the Relationship between the units used to express retinol and carotene and the Inorganic constituents. This part deals with the variability of nutrients in foods, the bioavailability of nutrients and the instructions for food labelling. The importance of the data, giving the weight changes on preparation of foods and the calculation methods of the composition of dishes prepared from recipes, should be stressed. The vitamin loss estimation in foods are rather important for the recipe calculations. The data of foods serve as bases for the Computerized Menuplanning Method in the nutrition. The data of foods, the calculation methods and the instruction tables are usable in both the nutrition and the food engineering and also in the food technology.

B. Gion

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RECENTLY ACCEPTED PAPERS

Enthalpy–entropy and frequency factor–activation energy compensation relations for death of *Escherichia coli* with microwaves in a tubular flow reactor
ÖZILGEN, S. & ÖZILGEN, M.

Enzymatic hydrolisis of carrot cell-wall polysaccharides, after isolation or in situ
MASSIOT, P., BARON, A. & DRILLEAU, J. F.

Changes of colour of the skin and the anthocyanin content of sour cherries during frozen storage
URBÁNYI, GY. & HORTI, K.

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References. The reference list (Literature) should be in alphabetical order as follows:

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Acta Alimentaria, Vol. 21 (3–4), pp. 195–203 (1992)

ENTHALPY – ENTROPY AND FREQUENCY FACTOR – ACTIVATION ENERGY COMPENSATION RELATIONS FOR DEATH OF *ESCHERICHIA COLI* WITH MICROWAVES IN A TUBULAR FLOW REACTOR

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Food Engineering Department, Middle East Technical University, 06531 Ankara. Turkey

(Received: 21 March 1991; accepted: 19 November 1991)

Kinetic analyses of death of *Escherichia coli* with microwaves in a tubular flow reactor were done by using the frequency factor – activation energy and activation entropy – activation enthalpy compensation relations. There was no actual isokinetic temperature for the death of *E. coli*, implying that the mechanisms of the death were different both in the microwave tubular flow reactor under varying experimental conditions and also in the typical constant temperature water bath. Although the death mechanisms appeared to be changing with the experimental conditions, the results implied that the compensation relations can be used in the design of the microwave pasteurization reactors.

Keywords: activation energy – frequency factor compensation, enthalpy – entropy compensation, death kinetics, microwaves

Death of the microorganisms generally follows first order kinetics (BAILEY & OLLIS, 1986):

$$\frac{dX}{dt} = -k_d X \tag{1}$$

Temperature dependency of the thermal death rate constant k_d may expressed with the Arrhenius expression as:

$$k_d = k_{ko} \exp(-\frac{E}{RT}) \tag{2}$$

Since the death of the cells may occur due to very large number of causes, numerical values of the constants k_d, k_{do} and E strongly depend on the experimental conditions. Rate constants of unimolecular chemical reactions may be predicted with the Eyring theory (DANIELS & ALBERTY, 1975). Using the analogy between the unimolecular chemical reactions and the thermal death kinetics as described in Equation 1, the numerical value of the death rate constant k_d may be estimated as:

$$k_d = \frac{\kappa RT}{N_A h} \exp(\frac{\Delta S^*}{R}) \exp(-\frac{\Delta H^*}{RT}) \quad (3)$$

Comparison of Eqn 2 with Eqn 3 ($\kappa \approx 1$) requires (DANIELS & ALBERTY, 1975):

$$E = \Delta H^* + RT \quad (4)$$

and

$$k_{do} = 2.72 \frac{RT}{N_A h} \exp(\frac{\Delta S^*}{R}) \quad (5)$$

Generally linear relations are observed between the activation energy E and the frequency factor k_{do}; and the activation enthalpy ΔH^* and the activation entropy ΔS^* of the family of related experiments:

$$\ln k_{do} = \alpha E + \beta \quad (6)$$

$$\Delta S^* = \delta \Delta H^* + \phi \quad (7)$$

Thermal death of *Escherichia coli* with microwaves in a tubular flow reactor under different flow conditions or with heat effects in a conventional constant temperature water bath may be considered as family of related experiments. The relations expressed by Equations 6 and 7 are referred to as the kinetic compensation relations and were widely observed in various areas of chemistry and biology (BARNES et al., 1969; LUMRY & RAJENDER, 1970; ELIZODO & LABUZA, 1974; UDEN & VIDAL-LEIRIA, 1976; RHIM et al., 1990). The kinetic compensation relations claim that in a family of related reactions the activation energy and the frequency factor and the activation enthalpy and the activation entropy were not independent from each other and in such reactions any change in the activation energy were compensated by the changes in the frequency factor; and also any change in the activation entropy were compensated with the changes in the activation enthalpy. Numerical values of parameter E indicates the sensitivity of the reaction rate constant to temperature variations, i.e. the larger the numerical value of parameter E, the larger is the variation of parameter k_d with temperature changes. In biological systems frequency factor – activation energy compensation relations may serve to reduce the sensitivity of the microorganisms to the temperature effects (LUMRY & RAJENDER, 1970; ELIZODO & LABUZA, 1974). Numerical values of parameters k_{do}, E, ΔS^*, and ΔH^*, depend on the reaction conditions such as velocity in a flow reactor, however the parameters α, β, δ and ϕ do not depend on the reaction conditions. All the reactions of a related family may have the same rate at a certain temperature. This is referred to as the isokinetic temperature (BARNES et al., 1969;

LUMRY & RAJENDER, 1970; ELIZODO & LABUZA, 1974; UDEN & VIDAL-LEIRIA, 1976; RHIM et al., 1990). When an isokinetic temperature exists, coefficients of Equations 6 and 7 may be expressed as $\alpha = 1/RT$, $\beta = \ln k_c$, $\delta = 1/T_c$ and $\phi = \Delta G^*/T_c$ (RHIM et al., 1990). While studying the kinetics of virus inactivation, BARNES and co-workers (1976) claimed that having an actual isokinetic temperature indicates that all the members of the related family of experiments were actually occuring with the same mechanism.

In our recent study of thermal death kinetics of *E. coli* with microwaves in a tubular flow reactor under different flow conditions or with heat effects in a conventional constant temperature water bath, the experimental results suggested that the death mechanism of *E. coli* with microwaves was not the same as its death mechanism with heat effects in the water bath (ÖZILGEN, 1990, ÖZILGEN & ÖZILGEN, 1991). In the present study enthalpy – entropy and frequency factor activation energy compensation relations will be sought for the death kinetics of *E. coli* and the criterion suggested by BARNES and co-workers (1969) will be used for obtaining more information about the death mechanisms.

1. Materials and methods

Glass tubular flow reactors with 7.5×10^{-3}, 9×10^{-3}, or 11.8×10^{-3} m outer diameter and 0.90, 1.50 and 2.00 m length were placed into the microwave oven (Vestel Goldstar, Model 5053 T, Turkey with 2450 MHz frequency) in parallel with the microwave source. The distance between the microwave source and the reactor was 0.14 m. A peristaltic pump (Millipore, USA) was used to pump the liquid medium to get constant average flow velocities of 0.031 m s^{-1}, 0.038 m s^{-1}, 0.045 m s^{-1}, 0.048 m s^{-1}, 0.060 m s^{-1} and 0.067 m s^{-1} in the reactor. Turbulent flow prevailed in the reactor with all the reported velocities.

Escherichia coli was isolated in our previous studies. Non-fat-dry-milk medium was purchasd in the local market and contained 1% fat, 35% proteins, 52% lactose, 8% minerals and balance water. *E. coli* was cultivated in 5% NFDM in shake flasks at 37 °C and a portion of this preparation was dispersed in 2×10^{-3} m^3 of 5% NFDM to obtain a medium inoculated with 10^5 cfu cm^{-3}. This preparation was pasteurized by passing through the reactors. The viable biomass concentrations were measured before and after leaving the pasteurization reactor.

Microbial concentrations were the averages of at least four plate counts on VRB agar (violet red bile agar, Oxoid, England). The temperature of the flow media was measured with thermocouples connected to a digital read-out (Nel, Model KS10, Turkey) before entering and after leaving the microwave oven. All the measurements were obtained after the system had reached the steady state. Inlet temperature of the inoculated medium to the reactor was about 294 K.

Temperature variations were described after making energy balance around the reactor (ÖZILGEN & ÖZILGEN, 1991) as:

$$\frac{dT}{dZ} = K_1 \exp(-\beta T) - K_2 (T - T_{env}) \tag{8}$$

where T and T_{env} were the temperature of the medium at location Z and the oven temperature, respectively. Parameter Z was the distance along the reactor measured from the entrance. Parameters K_1, K_2 and β were constants (K_1 = 2000 ± 20)/v (°C m^{-1}) where v = average flow velocity, K_2 = 0.69 ± 0.01 (1/m), β = 0.00012 (1/°C) and T_{env} = 293 K. Temperature variations along the reactor were simulated after substituting the values of the constants and solving Equation 8 numerically. Details of the solution were described elsewhere (ÖZILGEN & ÖZILGEN, 1991).

Equations 1 and 2 were used for describing death rates of *E. coli* along the reactor. Numerical values of the frequency factor k_{do} and the activation energy E were determined by trial and error procedure. First values of parameters E and k_{do} were assumed, the death rate constant k was calculated for each section of the reactor by employing the simulated temperatures and Equation 2, then fraction of the surviving microorganisms leaving that section were calculated with Equation 1 (section = 0.01 m reactor segment). The trial and error process was stopped when sum of the square error difference between the experimentally determined fractions of the surviving *E. coli* cells (at Z = 0.90 m, Z = 1.50 m and Z = 2.00 m) and their simulations were minimum. Details of the simulations were described elsewhere (ÖZILGEN & ÖZILGEN, 1991).

The inoculated medium was pasteurized also in the test tubes in a typical bench-top constant temperature water bath at 326 K, 330 K, 333 K and 337.5 K. Microorganisms were counted by the same method as explained earlier. Death rate constants were calculated by using the standard procedures with the integrated form of Equation 1. Activation energy E and the preexponential constant k_{do} were calculated from Equation 2 by making Arrhenius plots. Details of these calculations were given elsewhere (ÖZILGEN & ÖZILGEN, 1991).

2. Results and discussion

Inlet temperature of the inoculated media to the reactor was about 294 K. Exit temperatures from the 2.00 m reactor varied between 332 K (when v = 0.067 m s^{-1}), and 358 K (when v = 0.031 m s^{-1}). Fraction of the surviving microorganisms at the end of the 2.00 m reactor varied between 0 (with v = 0.031 m s^{-1}) and 0.84 (with v = 0.067 m s^{-1})(Table 1). Detailed information about the variation of the temperature and the viable fraction of the microorganisms along the reactor were

given elsewhere (ÖZILGEN & ÖZILGEN, 1991). Parameters ΔH^* and ΔS^* were calculated with Equations 4 and 5, respectively. Although these equations imply that ΔH^* and ΔS^* were functions of temperature, in the range of the experiments where temperature changed from 294 K to 358 K, less than 5% variation was observed in these parameters, therefore constant values were reported in Table 1.

Table 1

Variation of the numerical values of the kinetic parameters with the experimental conditions

Pasteurization system and experimental conditions	k_{do} (1/s)	E (J mol^{-1})	ΔH^* (J mol^{-1})	ΔS^* [J(K mol)$^{-1}$]
MW reactor OD = 0.0075 m v = 0.067 m s^{-1} T = 332 K x/x_0 = 0.84	5.89×10^{10}	8.1×10^4	7.8×10^4	−48.0
MW reactor OD = 0.0075 m v = 0.060 m s^{-1} T = 338 K x/x_0 = 0.38	3.23×10^{11}	8.3×10^4	8.0×10^4	−34.0
MW reactor OD = 0.0075 m v = 0.045 m s^{-1} T = 348 K x/x_0 = 0	5.86×10^{12}	9.1×10^4	8.8×10^4	−10.1
MW reactor OD = 0.0075 m v = 0.038 m s^{-1} T = 353 K x/x_0 = 0	1.95×10^{13}	9.4×10^4	9.1×10^4	−0.3
MW reactor OD = 0.009 m v = 0.048 m s^{-1} T = 338 K x/x_0 = 0.28	1.31×10^{12}	8.6×10^4	8.3×10^4	−22.3
MW reactor OD = 0.0118 m v = 0.031 m s^{-1} T = 358 K x/x_0 = 0	1.59×10^{24}	9.4×10^4	9.1×10^4	−1.4
Test tube	3.81×10^{24}	17.0×10^4	16.7×10^4	216.3

MW reactor: Microwave reactor

OD : Outer diameter of the tubular reactor

T : Temperature at the exit of 2.00 m reactor

x/x_0 : Fraction of the surviving microorganisms at the end of the 2.00 m of reactor length

Values of k_{do} and E were determined with trial and error procedure and reported in Table 1. Changing the tabulated values even with ±1% caused substantial increase in the sum of square error between the experimental data and the model, therefore the error associated with the constants reported in Table 1 may be considered less than ±1%.

Numerical values of the parameters α, β, δ and ϕ of Equations 6 and 7 were calculated by linear regression. The regression lines were plotted in Figs. 1 and 2 and compared with the experimental data. The apparent isokinetic temperature and death rates for *E. coli* were calculated from relations $\alpha = 1/RT_c$, $\delta = 1/T_c$ and $\beta = \ln k_c$ as $T_c = 346$ K and $k_c = 0.092$ s^{-1}; however substituting the isokinetic temperature T_c into Equation 2 yielded almost three folds of difference in the predicted values of the death rate constant k_c. These results indicated that the death mechanism of *E. coli* was not the same with all of the experiments of Table 1. Neglecting the experimental results obtained with the constant temperature water bath changed the numerical value of the apparent isokinetic temperature as $T_c = 300$ K, but the death rate constants of the remaining experiments were still

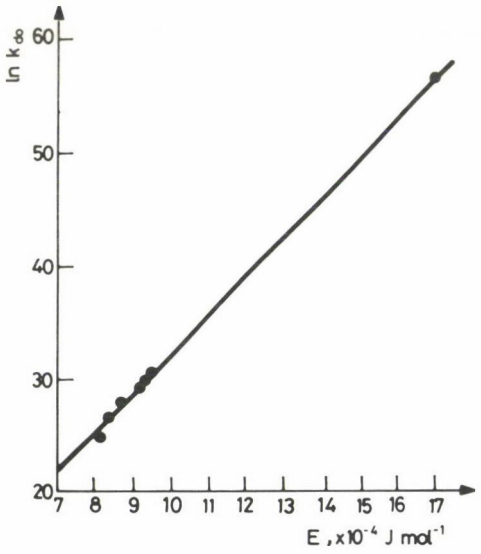

Fig. 1. Variation of the frequency factor ln k_{do} with activation energy E under different experimental conditions. Equation of the line: ln $k_{do} = -2.39 + 3.48 \times 10^{-4} E$ (correlation coefficient = 0.99)

about two folds different at this new isokinetic temperature. According to the criterion of BARNES and co-workers (1976) this result implies that *E. coli* had different death mechanisms when subjected to heat in the test tubes in a constant

temperature bath and in the microwave reactor. The results also indicated that even in the microwave oven more than one death mechanisms were prevailing. The cellular structure of the bacterial cells are more sophisticated than the structure of the viruses, observation of different death mechanisms under slightly varying experimental conditions may be caused by this sophisticated structures.

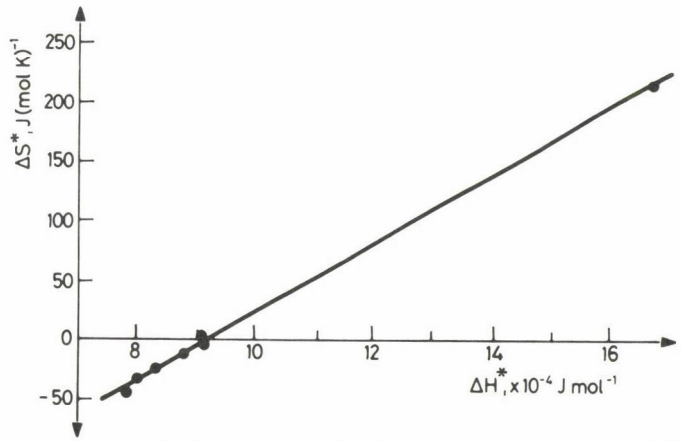

Fig. 2. Variation of the activation entropy ΔS^* with the activation enthalpy ΔH^* under different experimental conditions. Equation of the line: $\Delta S^* = -265 + 2.89 \times 10^{-3} \Delta H^*$ (correlation coefficient = 0.99)

According to the Eyrings theory (DANIELS & ALBERTY, 1975) first an activated complex is formed from the reactants, then the activated complex is converted into the reaction products. Numerical values of the activation entropy ΔS^* indicate the amount of the structural changes that the reactants undergo while forming the activated complex. In chemical reactions numerical values of ΔS^* usually vary between -100 and 100 J mol^{-1} (DANIELS & ALBERTY,1975). If there is an increase in the rotational and vibrational freedom of the activated complex ΔS^* is positive, if there is a decrease in rotational and vibrational freedom of the activated complex ΔS^* has a negative value (DANIELS & ALBERTY, 1975). It was seen in Table 1 that numerical values of the activation entropy ΔS^* were negative in all the experiments with microwaves, whereas the numerical value of the activation entropy was positive in the experiments with the constant temperature bath. These results may imply that different activated complexes were formed in both systems. In the microwave fields the molecules loose their vibrational freedom and try to align with the microwave field (GOLDBLITH, 1966). Having negative values for the activation entropies may show the effects of the microwave fields on the microbial species.

Although the results have indicated that there was no actual isokinetic temperature for death of *E. coli* with microwaves, the compensation relations can

still be used in the design of the microwave pasteurization processes. In chemical rections the term "mole" applies to the actual moles of the chemical species but in microbial systems the term "mole" is an apparent, i.e., empirical, term which may be obtained from the elemental analysis of the cell.

3. Conclusions

Parameters associated with thermal death kinetics of *E. coli* agreed with the compensation relations. Analysis of the data indicated that there was no actual isokinetic temperature of death implying that the microorganisms died with different mechanisms both with varying experimental conditions in the microwave fields and in the constant temperature water bath. Inspite of the different death mechanisms the compensation relations can still be used in the design of the microwave pasteurization reactors.

Nomenclature

E: Activation energy (J mol^{-1})
ΔG: Activation Gibbs free energy (J mol^{-1})
ΔH: Activation enthalpy (J mol^{-1})
h: Planck constant (J s)
k_c: Isokinetic reaction rate constant (s^{-1})
k_d: Death rate constant (s^{-1})
k_{do}: Frequency factor (s^{-1})
N_A: Avogadro constant (mol^{-1})
R: Gas constant [J(mol K)$^{-1}$]
ΔS^*: Activation entropy [J(K mol)$^{-1}$]
T: Temperature (K or °C)
t: Time: (s)
T_c: Isokinetic temperature (K)
v: Velocity of the medium in the rector (m s^{-1})
x: Biomass concentration (cfu cm^{-3})
x_0: Biomass concentration at the beginning of the death process (cfu cm^{-3})
α: Constant in Eqn 6
β: Constant in Eqn 6
δ: Constant in Egn 7
ϕ: Constant in Eqn 7
κ: Transmission coefficient

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EFFECT OF EMULSIFIERS ON THE PROPERTIES OF PASTA PRODUCTS

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Pasta products from the flour of the *Tr. aestivum* variety Yubileynaya 50 were proposed with the emulsifiers Amidan 250 and Dimodan PM Hydrate and their mixture (1:1) instead of eggs.

Cooking properties of the pastas were determined and a sensory evaluation was performed.

Emulsifier action on cooking properties and sensory characteristics was optimum when applied at a concentration of 0.6%.

The chemical changes occurring with regard to the gluten and gliadin proteins upon the action of the emulsifiers were followed by solvent fractionation and size exclusion HPLC chromatography of the fractions as well as with SDS-PAGE analysis.

HPLC and SDS-PAGE analyses confirmed that fraction distributions were altered both by the action of emulsifiers and by cooking. The fractions soluble in 1 mol l^{-1} urea show the appearance of high molecular bands and the greatest changes. The distribution of SDS-soluble gluten protein fractions shows changes upon the action of β-mercaptoethanol treatment: the amount of low molecular fractions increases. This suggests the presence of S bridges.

The individual emulsifiers act – according to their nature – in different ways on the individual fractions. The greatest changes can be brought about by mixtures of the emulsifiers. It is concluded that the changes occurring in the pasta structure can be followed and partly interpreted by electrophoretic and HPLC chromatographic investigations.

Keywords: pasta structure, emulsifiers, cooking properties, electrophoretic and HPLC investigations

Mechanical properties of pasta products are effectively influenced by substances with surface active properties. These control, on one hand the water balance between gluten and starch and, on the other they promote a uniform distribution of fats.

MEUSER (1979) and SCHUSTER (1984) established that emulsifiers act partly on the surface or interact with the components of flour, proteins, lipids and carbohydrates. During preparation of the dough interactions occur between the emulsifier and the cereal protein giving rise to the formation of hydrophobic bonds, hydrogen bridge bonds and electrostatic interactions. The basic protein skeleton with its covalent bonds, is not interacting with the emulsifiers, the interaction is only prevalent with the amino acids bound to the side chain groups of the protein. The

nature of the bonds formed depends on the polarity of the side chain, the structure of the lipid and the pH-value of the system. For each protein-lipid combination there is an advantageous kind of bond whose ratio within the total bonds is higher than other kinds of bonds. MÖHR and KROLL (1986) studied the interaction between emulsifiers and proteins. Their results showed that an interaction of hydrophilic character occurs between added casein, gliadin and the emulsifiers. They found that non-ionic emulsifiers bind to the gliadin fraction and give rise to a complex interaction. The interaction between glutenin and the emulsifier is weaker than that between gliadin and the emulsifier, and is mainly of hydrophobic character. Between gliadin and the emulsifier hydrophilic interactions are given preference. Therefore these authors recommend a mixture of emulsifiers for influencing the properties of systems containing gliadin and glutenin.

Our experiments were aimed at studying the influencing effect of emulsifiers on dough structure and establishing, how this effect manifests itself in the changes in dough quality. We wanted to elucidate the changes occurring upon the action of emulsifiers by using solvent fractionation followed by size exclusion chromatographic HPLC analysis and SDS-PAGE investigation of the fractions.

1. Materials and methods

Flour of 200–250 μm granule size obtained from the *Tr. aestivum* variety Yubileynaya 50 was used throughout the experiments. Amidan 250B and Dimodan PM Hydrate (Grindstedt, Denmark) as well as their mixture (1:1) were applied as emulsifiers. The dough prepared without emulsifier was considered as control.

1.1. Preparation of experimental samples

A moisture content of 34% was taken into account in calculating the amounts of flour and water, respectively, for dough preparation. Emulsifiers were applied at a rate of 0–0.8% as calculated on flour mass.

A suspension or colloid solution was prepared from the emulsifiers with an aliquot part of the water at 40 °C, or these were mixed with the flour in the solid state. The flour was placed in a kneading machine, type Kneading Machine Impastatrice 2502. Then the emulsifier was added with the aqueous phase, or the necessary amount of water was added, and kneading was carried on for 15 min. After kneading, a small loaf was formed by hand from the dough, and the dough obtained was elastic. The dough was spread with a domestic type pasta-machine, type Mercato Atalas. A piece of 2 mm thickness, 20 cm length and 5 mm width was prepared from the spread dough (according to the cutting profile).

The dough was put on a filter paper and dried at 37 °C and 87% relative humidity for 24 h. This was followed by an afterdrying period of 48 h at room temperature.

1.2. Cooking trial and sensory qualification

Wet weight, water amount taken up during cooking as well as sensory properties after cooking were determined by a cooking test.

1.2.1. Method of cooking test according to KARÁCSONYI (1970). Two hundred and fifty cm^3 tap water were boiled in a high, 500 cm^3 beaker on an electric cooker (LP-300 VEB, Berlin, 1200 W output); 25 g intact pasta, free of debris were weighed on a counter-balance and then cautiously poured into the water, whereby the water was kept boiling moderately during 10 min. In order to prevent catching, the contents of the beaker were stirred, at the beginning of cooking, several times with a glass rod.

In order to determine the wet weight of the cooked pasta, it was washed and rinsed on a Buchner funnel connected to a vacuum source. After cooling, the pasta was weighed in a vessel of known weight. Weighing of the wet mass was followed by sensory evaluation.

Water uptake (V) in % is calculated as follows:

$$V = \frac{(b-a)}{a} \times 100$$

b: mass of wet pasta (g)

a: pasta taken for cooking (g)

The cooking and rinsing water obtained in the cooking test was concentrated by evaporation, then dried to constant mass at 105 ± 1 °C. For 25 g past, cooking loss (F) in % is calculated as follows:

$$F = a \times 4$$

F: cooking loss (%)

a: mass of cooking and rinsing water after concentration (g).

1.2.2. Sensory assessment according to the HUNGARIAN STANDARD (1986). The pasta product was assessed first in the raw (uncooked) then in the cooked state whereby cooking was performed according to the cooking test. Sensory assessment included four groups of properties:

- outer appearence
- aroma
- taste
- cooking properties, consistency.

Sensory evaluation was performed by a jury of 3 members. The maximum score for the individual groups of properties was 5. From the means within the individual groups of properties weighted mean values were calculated. Calculation of the weighted mean was perfomed as follows: Outer appearance × 1.1 + aroma × 0.7 + taste × 0.9 + cooking properties × 1.3.

1.3. Solvent fractionation

The pasta samples were examined after 15 and 30 min of cooking, respectively. The boiled pasta samples were dried at 60 °C and ground to flour size particles in a Lab Mill 1 QC – 114 type grinder. The principle of solvent fractionation is shown in Fig. 1.

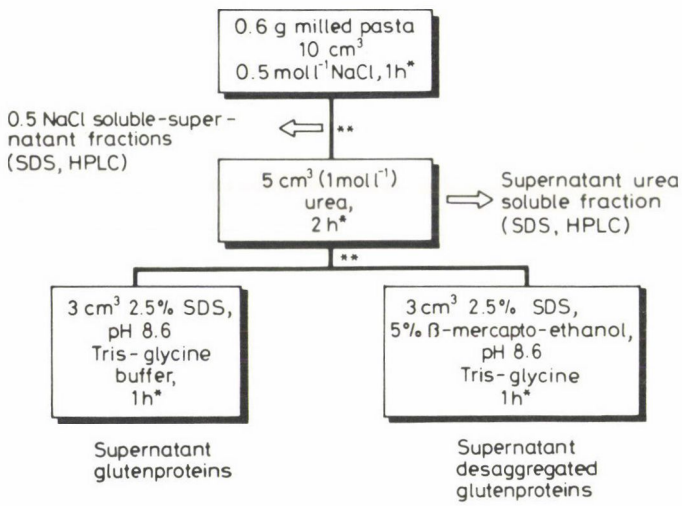

Fig. 1. Principle of fractionation. *Labor MIM magnetic stirrer; **Janetzki S30 centrifuge, min 4000 r.p.m.

In these experiments the respective amount of salt-soluble fractions were 10 – 15%, those of urea-soluble and SDS-soluble fractions were 30 – 35 and 35 – 40%.

1.4. HPLC fractionation of the samples

All the extracted samples were chromatographed using the following chromatographic parameters and equipment:

Chromatograph: Labor MIM HPLC
Column: Beckmann TSK 2000
Eluent composition: 0.2 mol l^{-1} NaH_2PO_4 + 2% SDS
Flow rate: 1 cm^3 min^{-1}
Detecting: 280 nm
Amount of sample applied: 20 μl.

The chromatograms obtained are compared and the molecular masses characteristic of the individual samples are established with the aid of the calibration curve. The equation of the calibration curve is as follows:

$$\lg \text{mol.mass} = 6.19 - 0.2879 \times X$$

X: retention time (s)
mol.mass (kD).

1.5. SDS-PAGE electrophoretic investigations

Electrophoretic investigations were carried out according to LAEMLI (1970). Proteins were separated in a 12.5% acrylamide gel. Twenty-fifty μl portions of the fractions were applied at one sampling point. A vertical gel electrophoretic apparatus was used. The size of the gel plates was 180 × 140 × 2 mm, the duration of electrophoresis was 16 h. During the pre-run current intensity was 10 mA, this was increased after 30 min to 50 mA. This value fell by the end of the run to 30 mA. The gels were fixed and stained in one step, in a mixture of 12.5% trichloroacetic acid and 0.25% Coomassie Brilliant Blue R-250 (19:É1). After 6 h the colour intensity of the bands was constant.

The molecular masses of the proteins separated by the SDS-PAGE method were estimated on the basis of the mobility and molecular mass of a protein of known molecular mass run on the sample plate.

2. Results

On the basis of earlier experiments it was established that emulsifiers could be applied at optimum concentrations. These are defined as concentrations where cooling losses are minimum, water uptake is high, sensory characteristics are optimum and consistency of the cooked pasta is unimpeachable. Table 1 shows the sensory and cooking properties of pasta products prepared with optimum emulsifier concentrations of 1%.

With respect to the cooking and sensory properties it can be established that cooking losses decrease upon the action of emulsifiers and are minimum when using a mixture of emulsifiers. Water uptake slightly decreases with the addition of emulsifiers and is minimum with the mixture of the two kinds. The cooking quality increases when using emulsifiers according to the control.

Table 1

Characteristic data of pastas prepared without (control) and with 0.6% concentration of emulsifiers

Characteristics	Control	Amidan 250B	Dimodan PM	Amidan 250B 1 Dimodan PM 1
Cooking loss (%)	12.40	7.70	8.53	5.29
Water uptake (%)	152.90	145.95	144.94	133.79
Outer appearance	5	5	5	5
Aroma (smell)	5	5	5	5
Taste	5	5	5	5
Consistency	4	5	5	5
Weighted mean of quality scores	18.70	20.0	20.0	20.0

Protein content (N×5.70) = 10.52%, moisture content = 9.21% (The data in the table represent the means of three measurements)

The distribution of molecular masss of 0.5 mol l^{-1} NaCl-soluble fractions is shown in Table 2.

The low molecular fractions of 2.8 and 0.8 kD of salt soluble fractions are slightly increased in raw pastas when emulsifiers are used.

Upon the effect of 15 min cooking, the ratio of 2.8–0.8 kD fractions considerably increases, and this indicates degradation of pasta structure. After 30 min cooking an increase in the amount of high-molecular fractions can be observed. An aggregation occurs in pasta structure, when the high molecular fractions appear (95.6–68.6 kD).

Considerable changes occur in the gluten protein fractions upon the effect of both cooking and emulsifiers. Two main fractions can be detected in raw pastas: 73.4 and 43.2 kD. The ratio of 1:3 of these changes to 1:2 on emulsifier action. Fifteen min cooking brings about the appearance of the high-molecular fraction of 95.6 kD. The different character of the emulsifiers becomes obvious with the appearance of fraction 37.8 kD. This fraction appears only when Dimodan PM is used, however the characteristic action of this emulsifier appears also in its mixture.

In excessively cooked samples only the high-molecular fraction of 95.6 kD can be detected in all treatments.

Table 2

Molecular mass distribution of 0.5 mol l^{-1} NaCl-soluble fractions in the percent of total protein
(HPLC-apparatus; Labor MIM, column: TSK 2000 Spherogel, Beckmann)

Sample		Mol.mass (kD) in % 95.6	68.6	37.8	8.8	2.8	2.18	0.8
Control	1	–	38.3	+	10.23	26.5	–	25.0
	2	+	7.6	+	–	16.1	40.0	36.1
	3	8.2	18.1	+	–	21.5	31.3	20.7
Amidan 250B								
0.6%	1	–	18.6	1.2	5.1	40.6	–	24.5
	2	+	12.6	+	+	19.5	44.8	23.0
	3	5.4	27.7	+	+	15.6	27.5	23.6
Dimodan PM								
0.6%	1	–	19.3	2.5	6.6	43.1	+	28.2
	2	1.8	19.0	3.2	1.7	21.8	25.0	27.2
	3	2.8	20.5	+	+	21.6	27.0	28.0
A:D = 1:1								
0.6%	1	–	16.8	2.0	3.68	44.8	–	32.5
	2	+	26.0	4.2	2.1	1.6	36.7	29.2
	3	6.3	26.2	+	+	1.2	44.8	21.8

1: raw pasta; 2: 15 min cooking; 3: 30 min cooking; +: traces

Table 3 shows the distribution of molecular weights in the fractions soluble in 1 mol l^{-1} urea.

Table 3

Molecular mass distribution of gluten protein fraction soluble in 1 mol l^{-1} urea in the percent of total protein
(HPLC-apparatus: Labor MIM, column: TSK 2000 Spherogel, Beckmann)

Sample		Mol.mass (kD) in % 95.6	73.4	43.2	37.8
Control	1	+	26.9	73.1	–
	2	94.0	+	–	6.0
	3	99.0	+	–	–
Amidan 250B 0.6%	1	+	38.4	61.6	–
	2	94.0	2.0	4.0	–
	3	98.0	2.0	–	–
Dimodan PM 0.6%	1	+	38.4	61.6	–
	2	32.3	7.6	–	60.0
	3	99.0	–	–	–
A:D = 1:1 0.6%	1	+	31.4	68.5	–
	2	53.7	4.3	–	41.2
	3	99.0	–	–	–

1: raw pasta; 2: 15 min cooking; 3: 30 min cooking; +: traces

Table 4 shows molecular mass distribution in SDS-soluble gluten protein fractions.

Table 4

Molecular mass distribution of SDS-soluble gluten protein fractions in the percent of total protein
(HPLC-apparatus: Labor MIM, column: TSK 2000 Spherogel, Beckmann)

Sample		Mol.mass (kD) in %							
		2.5% SDS TRIS-glycine pH = 8.6				2.5% SDS 5% β-mercaptoethanol TRIS-glycine pH = 8.6			
		95.6	68.6	40.0	10.2	95.6	68.6	40.4	10.2
Control	1	–	15.0	84.5	–	–	54.0	45.0	–
	2	+	63.0	18.0	18.0	+	63.0	18.0	16.0
	3	+	60.0	19.0	20.0	+	39.8	+	60.2
Amidan 250B 0.6%	1	–	15.0	84.5	–	–	52.6	47.3	–
	2	+	40.6	35.5	22.7	+	52.8	47.1	–
	3	+	40.2	36.0	22.5	39.0	41.0	9.8	10.2
Dimodan PM 0.6%	1	–	14.5	86.0	–	–	57.4	42.5	–
	2	+	40.0	36.0	24.0	+	75.0	12.0	12.5
	3	45.0	50.0	5.0	–	42.0	40.0	13.0	13.0
A:D = 1:1 0.6%	1	–	15.0	85.0	–	–	53.3	46.6	–
	2	+	76.0	12.0	11.8	+	80.0	10.0	9.5
	3	49.0	50.5	–	–	90.0	+	5.0	5.0

1: raw pasta; 2: 15 min cooking; 3: 30 min cooking; +: traces

Fraction distribution in raw pastas does not differ considerably from those in pastas prepared with emulsifiers. Upon the action of 15 min cooking the ratio of SDS-soluble low-molecular fractions increases, and this increase is further enhanced by β-mercaptoethanol treatment which is active towards S-bridges.

Excessive cooking of 30 min leads to the appearance of high molecular fractions whose distribution is only slightly changed upon β-mercaptoethanol treatment.

Figure 2 shows the electropherogram of the salt-soluble fraction as obtained by the SDS-PAGE method.

From Fig. 2 it can be seen that raw pasta contains the highest number of extractable fractions. No considerable difference can be observed between control and emulsifier-complemented pastas with respect to molecular mass distribution. After a cooking period of 15 min only the pastas containing emulsifiers show one or two characteristic bands. After 30 min cooking no protein fraction could be detected by electrophoresis.

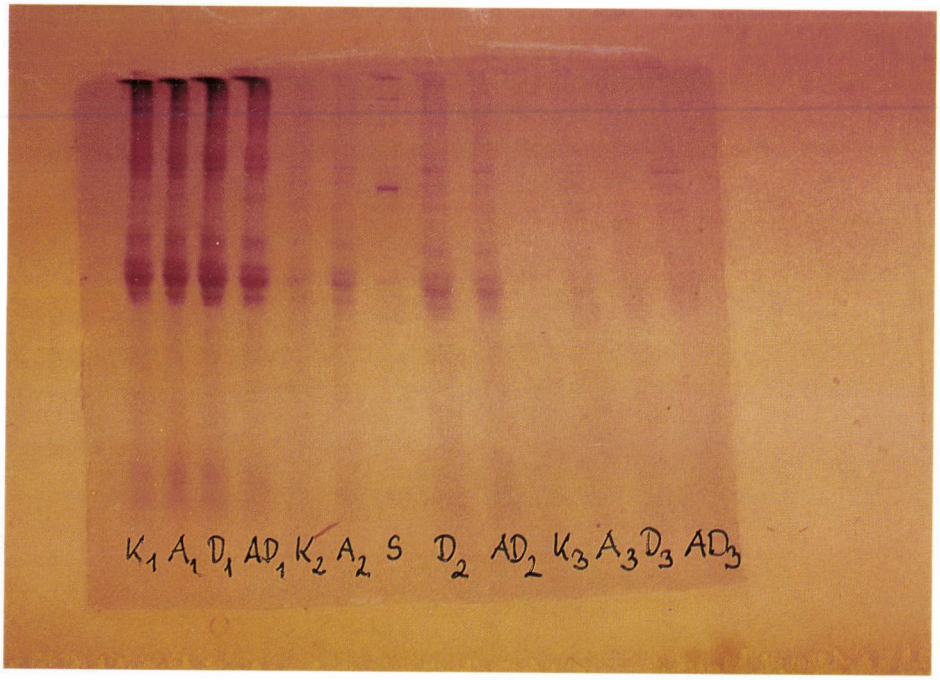

Fig. 2. Electropherograms of 0.5 mol l^{-1} NaCl-soluble fractions. Vertical gel electrophoresis apparatus, 12.5% acrylamide concentration, 2 mm gel plate; 50 mA, 16 h; staining with 0.25% Coomassie Brilliant Blue R-250. 1: raw pasta; 2: 15 min cooking; 3: 30 min cooking; K: control; A: Amidan 250B, 0.6%; D:Dimodan PM Hydrate, 0.6%; AD: Amidan 250B:Dimodan PM Hydrate (1:1), 0.6%. Standard proteins: albumin (67 kD), ovalbumin (43 kD), anhydrase (33 kD)

Figure 3 shows the SDS-PAGE result for the proteins extractable with 1 mol^{-1} urea.

The urea-soluble fractions show a similar molecular mass distribution for raw pastas. After 15 min cooking well separated fractions could be detected only in emulsifier-containing pasta but not in the control. After excessive boiling for 30 min no characteristic fractions were found.

Figure 4 shows the electropherograms of SDS-soluble fractions, while Fig. 5 shows the action of β-mercaptoethanol beside SDS.

From Fig. 4 it can be seen that SDS does not bring about noteworthy differences between the fractions, high molecular fractions can be detected even after 30 min excess boiling. β-mercaptoethanol treatment leads to an increase in low molecular fractions as shown in Fig. 5. With both SDS-fractions unambiguously detectable fractions appear after 15 and 30 min boiling, respectively.

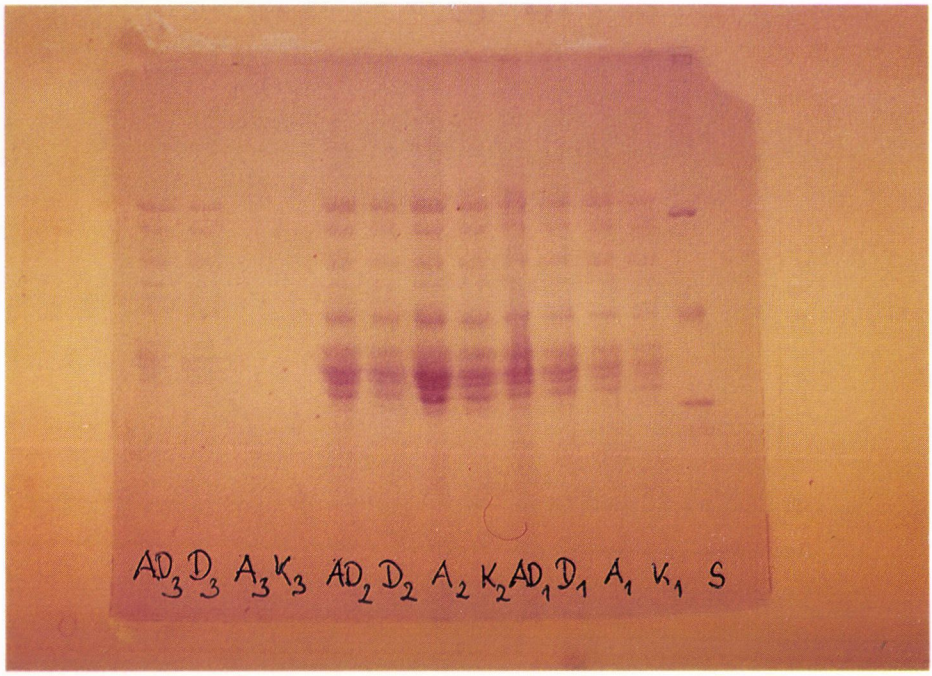

Fig. 3. Electropherograms of the 1 mol l^{-1} urea-soluble gluten protein fractions. Vertical gel electrophoresis apparatus, 12.5% acrylamide concentration, 2 mm gel plate; 50 mA 16 h; staining with 0.25% Coomassie Brilliant Blue R-250. 1: raw pasta; 2: 15 min cooking; 3: 30 min cooking; K: control; A: Amidan 250B, 0.6%; D: Dimodan PM Hydrate, 0.6%; AD: Amidan 250B:Dimodan PM Hydrate (1:1), 0.6%. Standard proteins: albumin (67 kD), ovalbumin (43 kD), anhydrase (33 kD)

3. Conclusions

Investigations carried out with control and emulsifier-containing pastas led to the following conclusions.

Emulsifiers promote the formation of dough properties, whereby final formation takes place during 15 min boiling of the pasta.

Emulsifiers decrease cooking loss and improve sensory properties of pastas, in the first place consistency.

Emulsifiers alter the distribution of molecular mass of the individual fractions, depending on their character. HPLC analyses make it possible to follow the degradation of pasta structure in salt soluble fractions as well as the aggregation taking place upon the action of excessive cooking.

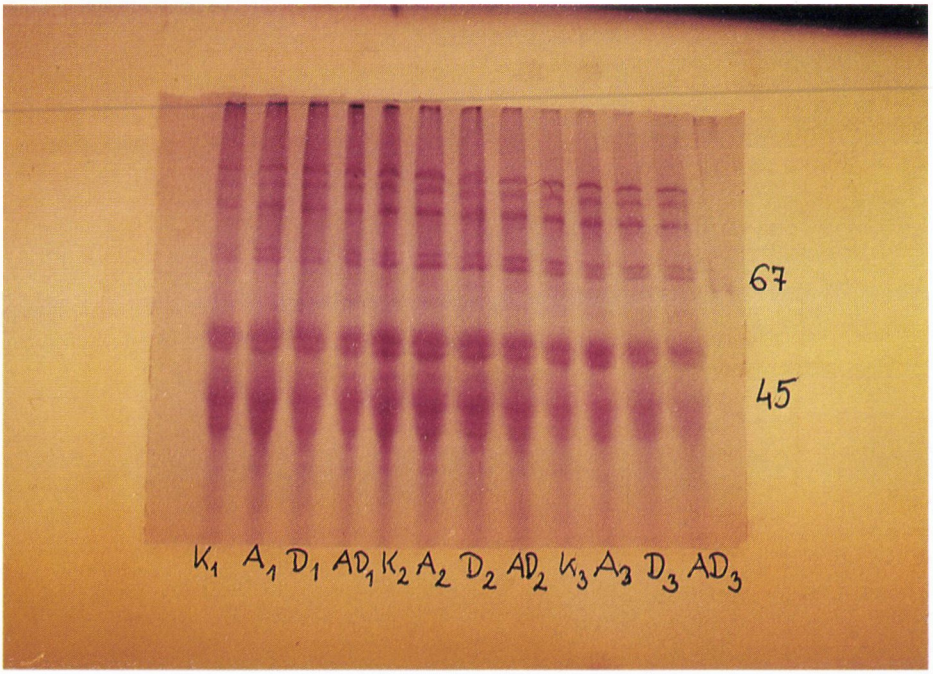

Fig. 4. Electropherograms of the SDS soluble gluten protein fractions. Vertical gel electrophoresis apparatus, 12.5% acrylamide concentration, 2 mm gel plate; 50 mA, 16 h; staining with 0.25% Coomassie Brilliant Blue R-250. 1: raw pasta; 2: 15 min cooking; 3: 30 min cooking; K: control; A: Amidan 250B, 0.6%; D: Dimodan PM Hydrate, 6%; AD: Amidan 250B : Dimodan PM Hydrate (1:1) 0.6%. Standard proteins: albumin (67 kD), ovalbumin (43 kD), anhydrase (33 kD)

According to HPLC-analyses the most important change occurs in the high molecular gluten protein fractions treated with 1 mol l^{-1} urea. Cooking leads unambiguously to the appearance of high molecular fractions, while after excessive cooking a single fraction could be observed only.

No considerable differences could be observed between the experiments with regard to SDS-soluble fractions by HPLC analyses, while the increase in low molecular fractions as brought about by β-mercaptoethanol indicates the presence of S-bridges in pasta structure.

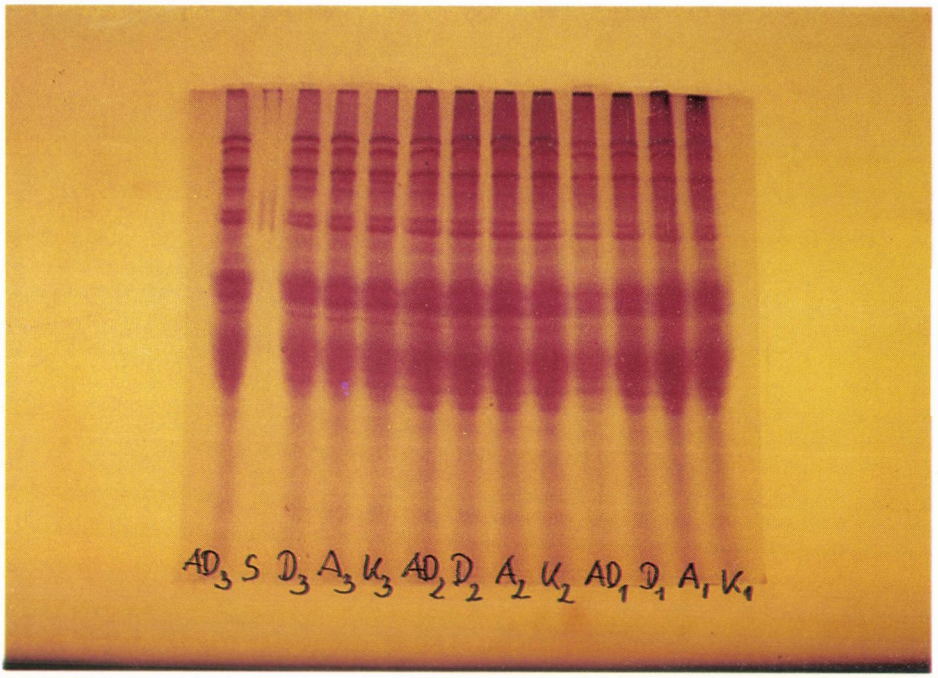

Fig. 5. Electropherograms of the SDS-soluble gluten protein fractions after treatment with β-mercapto-ethanol. Vertical gel electrophoresis apparatus, 12.5% acrylamide concentration, 2 mm gel plate; 50 mA, 16 h; staining with 0.25% Coomassie Brilliant Blue R-250. 1: raw pasta; 2: 15 min cooking; 3: 30 min cooking; K: control; A: Amidan 250 B, 0.6%; D: Dimodan PM Hydrate, 0.6%; AD: Amidan 250B : Dimodan PM Hydrate (1:1), 0.6%. Standard proteins: albumin (67 kD), ovalbumin (43 kD), anhydrase (33 kD)

SDS-PAGE investigations showed that the number of salt soluble fractions considerably decreases during 15 min boiling, and after 30 min no fraction is detectable at all. Urea-soluble high molecular fractions still show characteristic band after 15 min. No great differences were found in SDS-soluble gluten protein. The increase in low molecular fractions can be detected also by electrophoresis in β-mercapto-ethanol treated samples, indicating the presence of S-bridges. The increase of the 10 low molecular fractions can be explained by rupture of S-bridges. These permit of the conclusion that the final formation of pasta structure from gluten takes place during boiling. The similar fraction distribution as obtained in electrophoretic investigations might be interpreted by the assumption that in the conditions of electrophoresis the interactions between emulsifier, proteins, carbohydrates and lipids cease to exist.

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PHYSICAL AND CHEMICAL EVALUATION OF PEANUT BUTTER DURING STORAGE

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Peanuts have been recognized as evaluable source of vegetable protein, therefore the acceptability, physical and chemical properties of peanut butter prepared using different formulas, were determined. The results showed that the addition of sugar, salt and lecithin improved the flavour and overall acceptability of peanut butter. Casein caused a remarkable increase in protein content when added to peanut butter. Minerals differ very slightly among the different peanut butter formulas, although calcium and phosphorus content of peanut butter increased slightly as a result of the addition of casein. The creaming stability was higher for the peanut butter containing casein. The addition of casein and lecithin increased the shelf life of peanut butter and caused a greater value of oxidative stablility which was determined as thiobarbituric acid (TBA).

Keywords: peanut butter, peanut butter components, physical and chemical properties of peanut

Peanuts (*Arachis hypogea* L.) are an important source of edible vegetable oil and vegetable protein (CONKERTON & ORY, 1976). Peanuts have been recognized as a valuable source of vegetable protein (HARRIS et al., 1972). BIRD (1975) reported that in the future, plant proteins will find a greater use in food products. He also predicted that one-third to one-half of food grade protein will be derived from plant sources. Peanuts are the source of peanut butter, which accounts for approximately half of the total food use of peanuts in the USA (PESURRECCION, 1988).

The lack of colour, bland flavour and low concentration of flatulence producing carbohydrates in peanuts make it more desirable choice of food supplements than other plant proteins (SMITH, 1972). A light coloured paste, preferably free of peanutflavour is desired in such a product, therefore peanut kernels were steamed as opposed to being roasted in order to prevent development of the brown colour and "roasted peanut" flavours that accompany the roasting process (OUPADISSAKOON, 1980). The purpose of heat treating certain foods is to promote flavour changes that ultimately increase the over all platability of the product. When heated accordingly by volatile oils common to all these products are produced which influence their aroma and taste characteristics. To acknowledge the effect of roasting on the sensory attributes would therefore not only be desirable, but necessary to maintain quality control and overall flavour acceptance (BUCKHOLZ et al., 1980) peanut products have been extensively evaluated using sensory analysis alone and in combination with instrumental analysis (DUPUY et al., 1974; YOUNG et al., 1976).

The salt which is added to peanut butter at level 1–2% of the final butter contribute to the flavour. Salt also acts as a preservative. The salt goes into a solution in the water droplets and there the salt concentration is then about 7 times the level of the salt added. At this concentration, salt is a strong preservative within the water droplet and largely prevents the growth of spoilage bacteria in these droplets (POTTER, 1973). Lecithin is a primary antioxidant and also acts as a synergist in virtue of the metal ion binding effect of its phosphoric acid group (BERK, 1976). Sugar is one of the principal ingredients which produce tenderness. Also when sugar was included in the recipe, rancidity was delayed (MEYER, 1978). Casein is a conjugated protein containing phosphoric acid, calcium and some carbohydrate, hence casein improves the texture and reduces rancidity when added to peanut butter (BERK, 1976).

1. Materials and methods

1.1. Materials

Peanuts (crop 1990) were purchased from a commercial market in Alexandria, packaged in low density polyethylene bags (1 kg each). The bags were stored in controlled environment at 4 °C.

Casein was used in one formula as a protein source, binding agent and stabilizer. It was bought from Alexandria company for extract oil.

Sodium chloride salt and sucrose were bought at the local market in Alexandria and were used in peanut butter to enhance the flavour.

Lecithin was added at a concentration of 0.1%, as an emulsifier and antioxidant (BERK, 1976).

1.2. Methods

1.2.1. Peanut butter preparation. Peanuts were soaked in hot water (80 °C) for 30 min, and were dehulled by hand. The dehulled peanuts were air dried at room temperature for 1 h. Then the dried peanuts were roasted in a laboratory oven (Rowenta, gourmet) for 25 min at 167 °C. The peanuts were divided into 5 batches as shown in Table 1. Then each batch of the peanuts was milled using Brown Electric mincer until obtaining homogenized peanut butter. The newly ground peanut butters were sealed in plastic bags and refrigerated over night before it was brought to room temperature after different storage times (15, 30, 45 and 60 days) (PATTEE et al., 1982).

Table 1

Formulation ingredient for the different peanut treatments

Ingredient of peanut	Formula code A	B	C	D	E
Peanut butter (g)	300	300	300	300	300
Casein (w %)	-	1.50	-	-	-
NaCl (w %)	1	1	1	1	1
Sucrose (w %)	-	-	2	-	2
Lecithin (w %)	-	-	-	0.1	0.1

1.2.2. Sensory quality evaluation. Sensory quality attributes of peanut butter samples were determined by a 10 member panel. Colour, flavour and overall acceptability were evaluated on the basis of 9 point scales (9 = excellent, 1 = very poor).

1.2.3. Chemical analysis. Moisture, crude protein (% N × 6.25), crude fat, ash and crude fiber were determined by A.O.A.C. (1980) procedures.

1.2.4. Mineral analysis. Samples of each peanut butter formula was ashed and analyzed for Ca, P, K, Na and Fe. Measurement of the elements was made on an PYE-Unicam Sp 1900 atomic absorption spectrophotometer according to EMMEL and co-workers (1977).

1.2.5. Evaluation of creaming stability of the emulsion. Creaming stability was evaluated by calculating the stability rating (SR) basically according to TORNBERG and HERMANSSON (1977) and YAMAUCHI and co-workers (1980). The peanut butter (60 g) was allowed to stand at 26 °C for 1 day in a glass tube (initial fat content : F_0). Then 3 cm^3 of the emulsion were carefully removed from the bottom of the tube and the fat content of this portion was determined (F_1). The residual emulsion was centrifuged at 180 × G for 10 min. Three cm^3 were then aspirated from the bottom of the tube and the fat content was determined (F_2). SR values were calculated as follows:

$$SR_1 = \frac{F_1}{F_0} \times 100(\%)$$

$$SR_2 = \frac{F_2}{F_0} \times 100(\%)$$

In this evaluation a stable emulsion gives a value near 100% while an unstable emulsion gives a lower value.

1.2.6. Oxidative rancidity. The extent of oxidative rancidity development was determined by the thiobarbituric acid (TBA) test of peanut butter prepared using different formulas immediately after preparation and after 15, 30, 45 and 60 days of

storage at room temperature. The distillation TBA method of TARLADGIS and co-workers (1960) was used.

2. Results and discussion

2.1. Sensory quality evaluation

The results in Table 2 evidence the significant differences found in both flavour, colour and overall acceptability of peanut butter of different formulas. Peanut butter coded *E* containing sucrose, lecithin and salt had the highest scores comparing with the other formulas. Storage time up to 45 days showed no significant ($P \leq 0.05$) effect on the peanut butter, especially, formulas *D* and *E* which contained lecithin. However, increasing the storage time up to 60 days caused a remarkable change in the flavour, colour and overall acceptability. Colour score decreased from 6.25 at zero time to 5.9 after 60 days of storage for peanut butter coded *A*. Same trend was observed for flavour and overall acceptability scores. PATTEE and co-workers (1982) reported no significant effect of the storage time upon roasted flavour or colour of peanut butter. However PESURRECCION (1988), found that premium brand peanut butter had significantly higher roasted flavour scores than the store brand samples.

2.2. Proximate composition

The proximate composition of peanut butter is shown in Table 3. Moisture content was significantly low at all the peanut butter formulas, this low moisture content was due to drying during the roasting process. Moisture content showed no change with the change in peanut butter formula. Peanut butter containing casein had the highest protein (28.2%) and lowest fat and carbohydrate content % (47.3%, 17.26%, respectively) in comparison with all the other peanut butter formulas.

2.3. Mineral content

As shown in Fig. 1. minerals differ very slightly among the different peanut butter formulas. Calcium and phosphorus content of peanut butter coded *B* were high compared with the others, this is due to the presence of casein (which is a good source of calcium and phosphorus) in peanut butter *B*.

Table 2

Mean of flavour, colour and overall acceptability scores of peanut butter after different storage periods

Storage time (day)	Colour					Flavour					Overall acceptability				
	A	*B*	*C*	*D*	*E*	*A*	*B*	*C*	*D*	*E*	*A*	*B*	*C*	*D*	*E*
0	6.25	7.11	7.38	7.95	8.15	6.80	7.30	8.10	7.93	8.01	6.26	7.31	7.93	8.12	8.52
15	6.91	7.39	7.85	8.21	8.15	6.71	7.12	7.75	7.80	8.00	6.82	7.61	7.95	8.31	8.50
30	6.05	7.05	7.53	8.15	8.04	6.52	7.00	7.62	7.25	8.20	6.50	7.60	8.01	8.20	8.60
45	6.10	6.95	7.75	7.58	6.10	5.70	6.89	7.60	6.81	8.10	5.20	7.10	7.80	8.00	5.51
60	5.90	6.90	7.10	8.01	8.15	5.85	6.90	7.13	6.52	8.00	5.80	6.70	7.50	8.10	8.45
M	6.22	7.08	7.52	7.98	7.72	6.32	7.04	7.64	7.26	8.08	6.12	7.26	7.84	8.15	7.92
SD	0.562	0.572	0.315	0.75	0.906	0.487	0.172	0.341	0.584	0.085	0.57	0.304	0.182	0.104	0.252

M: mean value

SD: significant difference

Table 3

Gross composition of different formulas of peanut butter

Sample code	Moisture	Protein	Fat	Carbohydrate	Fiber	Ash
A	1.6±0.141	26.50+0.707	48.91+0.248	16.69+0.283	2.40+0.184	3.92+0.113
B	1.6+0.212	28.20+0.636	47.30+0.353	17.26+0.226	2.12+0.106	3.52+0.127
C	1.7+0.169	25.93+0.467	47.63+0.318	18.25+0.248	2.82+0.120	3.65+0.132
D	1.8+0.184	26.27+0.481	48.19+0.361	17.83+0.198	2.30+0.134	3.61+0.148
E	1.7+0.177	25.95+0.523	48.01+0.445	18.14+0.304	2.17+0.113	3.49+0.136

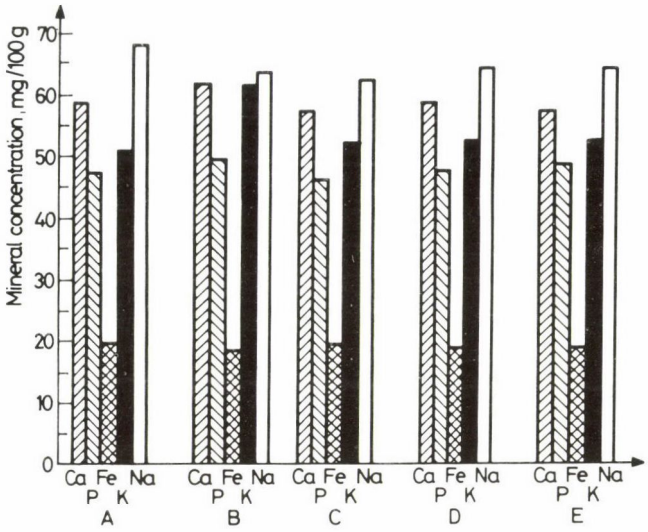

Fig. 1. Minerals content of different formulas of peanut butter

2.4. Creaming stability of peanut butter

Peanut butter is an emulsion of oil and water. Determining the creaming stability of peanut butter as shown in Table 4, revealed that peanut butter containing casein (*B*) had the highest creaming stability. Previous work proved that creaming stability was positively correlated with protein concentration (YAMAUCHI et al., 1980). Addition of lecithin as in peanut butter formulas *D* and *E* had no significant effect on the creaming stability. The data in Table 4 also demonstrate a gradual decrease in creaming stability with increasing time of storage.

2.5. Oxidative rancidity

TBA value of peanut samples at different storage times are presented in Table 5. The results revealed that storage time had a significant effect on increasing the oxidative rancidity of peanut butter. The greater loss in oxidative stability with storage time of peanut butter might be related to their significantly higher level of unsaturated fatty acids (PATTEE et al., 1982).

Addition of casein as in sample *B* and addition of lecithin as shown in samples *D* and *E* prevented relatively the rancidity development in peanut butter during storage time. This may be explained by the effect of phosphoric acid in lecithin which is capable of reinforcing the activity of some of the phenolic antioxidants and so act as a synergist (MEYER, 1978).

Table 4

Effect of storage time on the creaming stability of peanut butter

Sample code	Storage time (day)									
	0		15		30		45		60	
	SR_1	SR_2	SR_1	SR_2	SR_1	SR_2	SR_1	SR_2	SR_1	SR_2
A	76.90	60.50	75.0	59.2	73.9	58.1	70.38	57.1	65.2	56.2
B	78.70	67.30	77.9	66.9	76.1	66.1	72.1	66.0	68.1	69.2
C	78.21	62.19	76.3	61.2	72.5	61.0	69.8	60.2	66.2	58.4
D	78.95	57.30	78.9	57.0	77.0	57.1	76.8	56.2	76.1	56.1
E	79.25	59.50	79.0	59.1	79.1	53.9	78.5	53.2	78.1	57.5

Table 5

TBA values as affected by the storage time of peanut butter formulas

Sample code	Storage time (day) 0	15	30	45	60
A	2.320	2.820	3.420	4.020	4.690
B	2.280	2.32	2.61	3.12	3.31
C	2.32	2.75	3.25	3.99	4.61
D	2.35	2.61	2.66	3.25	3.35
E	2.30	2.57	2.69	3.29	3.28
M	2.114	2.614	2.926	3.534	3.35
SD	0.0267	0.193	0.3792	0.4346	0.735
SE	0.0116	0.086	0.1696	0.1943	0.3278

In conclusion peanut butter is a good source of plant protein and addition of casein enhanced the nutritive value as well as increased the keeping quality of the product.

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BIOCHEMICAL, RHEOLOGICAL, COOKING QUALITY AND ACCEPTABILITY OF DEFATTED SOY-SUPPLEMENTED WHOLE DURUM MEAL NOODLES

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Noodles were prepared from 100% commercial durum semolina (DS), 100% whole durum meal (WDM) and WDM supplemented with 5.0, 10.0 and 15.0% commercial defatted soy flour (DSF) to increase the protein quantity and quality. WDM had significantly higher protein, ash, fat and carotene contents and provided doughs of stronger mixing properties and quick-cooking noodles with a significantly higher swelling, firmness and tolerance to overcooking, but with greater cooking loss than the noodles made from pure DS. Fortification of WDM with DSF significantly increased protein, ash, fat, carotene, dough mixing strength, pasta firmness and cooking loss, but decreased swelling and tolerance to overcooking compared to the not fortified product. Sensory evaluation of WDM and WDM-fortified noodles compared favourably with those prepared from 100% DS or with standard commercial products containing 2 eggs and 4 eggs. All experimental noodle samples had a dark brown colour. Fortification increased the flavour, especially at 10% or more DSF. The overall acceptability scores were less desirable when DSF was increased to 10 or 15%, but WDM noodles containing 5% DSF was generally preferred over the unfortified WDM noodles. Production of new economical high protein pasta products using the native WDM supplemented with DSF up to 5% will significantly help to overcome some Egyptian domestic problems, such as the shortage of the technological means of semolina purification and the protein deficiency in the diet of low income groups.

Keywords: durum wheat, soy flour, pasta quality, soy-supplemented pasta

Recently in Egypt some high quality durum wheat varieties are cultivated in relatively wide areas. However, their utilization for manufacturing high quality pasta products is still impossible, because of the shortage of the technological means necessary for durum wheat milling and purification of semolina. Nevertheless, it is easy to obtain WDM using the technological facilities available in Egypt.

From biochemical and nutritional points of view, WDM is rich in protein, ash, dietary fiber, fat, minerals and vitamins, compared to the pure DS because considerable amounts of these components are concentrated in bran layers and germ separated during milling and semolina purification process. On the other hand, high protein content of raw materials is the main factor responsible for superior cooking quality of pasta products (MATVEEF, 1966; IRVINE, 1971; DAMIDAUX & FEILLET, 1978; DEXTER & MATSUO, 1980; TAHA & SÁGI, 1986). However, high contents of

protein and ash lead to the deterioration of pasta colour (IRVINE, 1971; KOBREHEL et al., 1974; TAHA & SÁGI, 1987).

Considering the above points, together with the economical situations and protein deficiency in the diet of low income groups in Egypt, production of new, economical, high protein foods, easy to prepare from native raw materials, may be significant in promoting good nutrition and good health for those people. Therefore, a high-protein pasta product was developed using WDM supplemented with DSF and evaluated in comparison with 100% DS as well as with standard commercial pasta products.

1. Materials and methods

1.1. Materials

WDM was obtained by dry milling of durum wheat using a laboratory mill. DS and pasta containing 2 eggs and 4 eggs served as controls (3 controls), respectively, DSF used for fortification was a commercial product available in Hungary.

1.2. Noodle processing

DSF was blended with WDM at levels of 0.0, 5.0, 10.0 and 15.0%. Noodle samples were processed using 5 kg of each blend as well as pure DS on a laboratory scale extruder at 50°C die and extrusion temperature and at a vacuum of 7.3 – 8 kP, respectively, in the Cereal Research Institute, Szeged, Hungary. Noodles were then dried at 40°C and 90% relative humidity for 1 h, followed by 14 h at 50°C and 80% relative humidity, then at 25°C and 66% relative humidity for the final 1.5 h.

1.3. Chemical analysis

Moisture, protein ($N \times 5.75$), ash and fat contents were determined as described in A.A.C.C. methods No. 44–15A, 46–11, 08–01 and 30–10, respectively (A.A.C.C., 1983). Carotene content was measured spectrophotometrically at 440 nm according to A.A.C.C. method (A.A.C.C., 1968).

1.4. Mixing test

Mixing properties of DS, WDM and WDM/DSF blends were determined by the ten-gram micro-mixograph technique of FINNEY and SHOGREN (1972), based upon the parameters dough development time (DDT), maximum consistency (MC) and total curve area (TCA).

1.5. Cooking quality

For the evaluation of cooking behaviour, 25 g of noodles were cooked in 250 cm^3 boiling water. Optimum cooking time, cooked weight, cooked volume and cooking loss % were determined according to the HUNGARIAN STANDARD (1958) method.

Firmness of cooked pasta and tolerance to overcooking were estimated after cooking for 15 and 30 min, respectively, using the aleurograph method of SCOTTI and co-workers (1976).

1.6. Sensory evaluation

Noodle samples processed from WDM and WDM supplemented with DSF and that from pure DS as well as with 2 eggs and 4 eggs standard commercial products were exposed to sensory evaluation by ten panel members (4 Egyptian, 6 Hungarian), based upon the parameters appearance, colour, surface case, odour, taste, mouthfeel and fragmentation according to HUNGARIAN STANDARD (1958) method.

1.7. Statistical analysis

Analysis of variance (LSD at 5%, *F* test) was carried out with a Commodore 64 personal computer, according to SVÁB (1981).

2. Results and discussion

2.1. Rheological properties

It is clear from Fig. 1 that based upon its mixogram, the dough prepared from WDM showed higher strength and higher stability during mixing compared to that prepared from DS, in spite of higher amount of water added (WDM : 6.5 cm^3, pure DS : 5.5 cm^3). It can also be seen that addition of DSF to WDM remarkably increases dough strength and stability during mixing.

Data presented in Table 1 revealed that addition of DSF at any level delayed the development of WDM dough and increased mixograph TCA. The strong mixing properties of DSF-supplemented WDM-doughs probably due to the higher protein content of these blends compared to that of pure DS or WDM (Table 2), since protein content showed significant positive correlation with mixograph DDT ($r = 0.98^{***}$, P = 1%) and mixograph TCA ($r = 0.87^{*}$, P = 10%) confirmed by earlier findings (TAHA et al., 1992a).

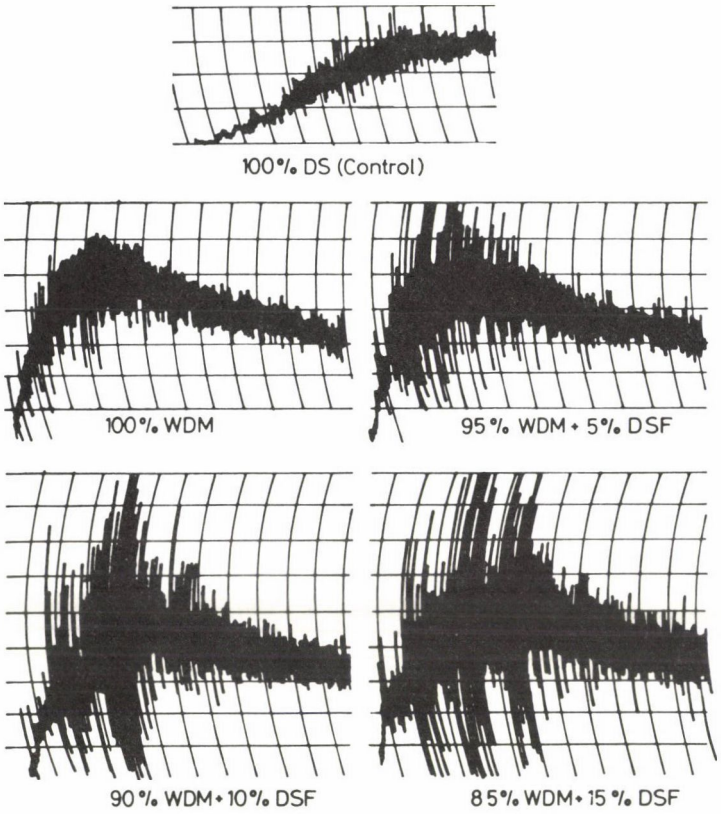

Fig. 1. Mixograms of doughs prepared from durum semolina (DS), whole durum meal (WDM) and whole durum meal + defatted soy flour (WDM + DSF) blends (10 g sample of 14% moisture, mixing time 7 min, water absorption 6.5 cm^3, except 5.5 cm^3 for 100% DS)

2.2. Chemical composition

As obvious in Table 2, noodles processed from 100% WDM contained about 1.3 times more protein, 2.3 times more ash, 4 times more fat and 1.6 times more carotene than the 100% DS noodles. This is due to the considerable amount of these components concentrated in bran and germ of wheat kernels separated from semolina during milling and purification. The pure DSF contained about 4.3 and 3.3 times more protein, 8.3 and 3.6 times more ash, 12 and 3 times more fat and 5.5 and 2 times more carotene than DS and WDM, respectively. These differences resulted in significant increases of protein, ash, fat and carotene contents of WDM-noodles

supplemented with DSF at all levels (Table 2). With increasing level of DSF supplementation, the protein, ash and carotene contents significantly increased and carotene processing losses decreased.

Table 1

Mixograph parameters of doughs prepared from durum semolina, whole durum meal and whole durum meal supplemented with defatted soy flour

DS(%)	Mixture description WDM(%)	DSF(%)	DDT (mm)	MC (MU)	TCA (cm^2)
–	100	–	36	580	15.2
–	95	5	39	535	15.7
–	90	10	49	553	20.0
–	85	15	54	589	36.4
100[a]	–	–	28	343	11.2
$LSD_{5\%}$			5.96	33.42	1.47
F test			72.2***	132.8***	125.4***

[a] control, DS: durum semolina, WDM: whole durum meal, DSF: defatted soy flour, DDT: dough development time, MC: maximum consistency, TCA: total curve area
LSD 5% = Less Significant Difference at P = 95% probability level
*** Very highly significant at P = 99.9% probability level

Table 2

Chemical composition of raw materials and noodles processed from durum semolina, whole durum meal and whole durum meal supplemented with defatted soy flour

Sample description			Moisture (%)	Protein (N × 5.75) (%)	Ash (%)	Fat (%)	Carotene (mg per kg) Crude mixture	Processed noodles		Carotene loss (%)	
Pure defatted soy flour					11.4	49.7	5.89	2.23	20.7	–	–
Noodles processed from:											
DS	WDM	DSF (%)									
–	100	–			8.8	14.9	1.63	0.74	10.6	6.02	43.2
–	95	5			9.0	16.7	1.92	0.91	10.8	6.61	38.8
–	90	10			9.1	18.0	2.06	0.83	10.4	6.93	33.5
–	85	15			8.2	20.3	2.20	0.89	10.9	8.35	23.6
100[a]	–	–			10.1	11.7	0.71	0.19	6.6	3.76	43.0
$LSD_{5\%}$ (for noodles)					0.10	0.85	0.07	0.13	0.54	0.07	4.02
F test (for noodles)					725.8***	228.9***	1256***	82.8***	180.2***	9513***	63.6

*** Very highly significant at P = 99.9% probability level
[a] control, DS: durum semolina, WDM: whole durum meal, DSF: defatted soy flour

Since Egyptian people, specially low income groups are accustomed to consume rice or pasta in large quantities as the main filling foods (the main dish), the supplemented new products represent a suitable way to increase protein intake and improve the nutritional value of the diet.

2.3. Cooking quality

Based upon the data reported in Table 3, optimum cooking time for noodles processed from both WDM and WDM fortified with DSF was shorter than for control DS noodles. However, reduction of cooking time by addition of DSF to WDM was significant only at the highest level (i.e at 15%).

Noodles processed from 100% WDM showed a significantly greater cooking loss than the control (100% DS) noodles. Fortification of WDM with DSF slightly, but significantly increased the cooking losses. Similarly higher cooking losses were reported for pasta products prepared from DS fortified with soy proteins (LAIGNELET et al., 1976) and soy flour (BUCK et al., 1987; TAHA et al., 1992b).

As demonstrated in Table 3, 100% WDM noodles showed a significantly higher cooked weight, cooked volume and firmness values of cooked pasta than those of 100% DS noodles. Fortification of WDM with DSF lead to a decreased cooked weight and cooked volume but to an increased firmness of cooked pasta in comparison with the values of unfortified WDM. Swelling index decreased and firmness values increased with increasing level of fortification except firmness of overcooked pasta at 15% DSF. These results confirm the previous studies of PAULSEN (1961). BUCK and co-workers (1987) on macaroni containing soy flour, LAIGNELET and co-workers (1976) on spaghetti enriched with soy proteins and TAHA and co-workers (1992b) on noodles fortified with full fat and defatted soy flours.

Percentages of firmness loss by overcooking (Table 3) revealed that WDM pasta had the highest tolerance to overcooking, but this tolerance has been significantly reduced by addition of DSF especially at 15% level.

2.4. Sensory evaluation

A comparison of data means obtained by sensory evaluation of noodles processed from pure DS and from commercial products containing 2 eggs and 4 eggs (3 controls), respectively, as well as of noodles processed from WDM and WDM supplemented with 5, 10 and 15% DSF is shown in Table 4.

Panel members gave the highest scores for the controls (without significant differences between them) for all parameters excepted odour test. Several panel members commented that pasta containing eggs had an objectionable odour.

Table 3

Cooking behaviour of noodles and firmness of cooked pasta made from durum semolina, whole durum meal and whole durum meal supplemented with defatted soy flour

Noodles components			Optimum cooking time	Swelling		Cooking loss	Firmness (aleurograph value in bar) Cooking time		Firmness loss by overcooking	
DS(%)	WDM(%)	DSF(%)	(min)	Weight (%)[a]	Volume (%)[b]	(%)[a]	15 min	30 min	Total	(%)
-	100	-	8.0	230	310	10.6	75	34	109	55
-	95	5	7.8	216	298	11.5	87	36	123	59
-	90	10	7.5	206	289	12.6	102	45	147	56
-	85	15	7.0	187	267	13.6	117	40	157	66
100[c]	-	-	14.8	209	289	6.7	61	25	86	59
$LSD_{5\%}$			0.82	5.83	8.96	0.48	2.50	1.29	3.48	2.09
F test			292.6***	189.9***	204.6***	324.6***	187.0***	180.6***	198.8***	178.7***

*** Very highly significant at P = 99.9% probability level

[a] on the uncooked noodles weight basis,

[b] on the uncooked noodles basis,

[c] control, DS: durum semolina, WDM: whole durum meal, DSF: defatted soy flour

Table 4

Sensory, evaluation of noodlees processed from durum semolina, whole durum meal and whole durum meal supplemented with defatted soy flour

Sample description			Maximal scores of: appearance 10	colour 10	surface case 15	odour 5	taste 20	mouth-feel 30	fragmentation 10	total 100	overall acceptability (%)
Noodles processed from:											
DS	WDM (%)	DSF									
-	100	-	5.2	3.7	10.0	4.3	13.4	20.8	8.4	66.8	73.0
-	95	5	5.5	4.4	10.6	4.5	13.3	22.8	8.0	69.3	75.6
-	90	10	5.8	4.1	9.4	3.6	13.0	22.6	8.1	66.6	72.3
-	85	15	6.5	4.0	9.1	3.3	10.9	21.6	7.9	61.3	63.7
100[a]	-	-	9.3	8.9	13.9	4.5	19.2	27.8	9.9	93.5	
Commercial products:											100
2-eggs 100% DS[a]			8.7	9.1	14.1	3.0	17.3	25.8	10.0	88.0	
4-eggs 100% DS			9.9	9.6	14.4	3.6	17.3	26.9	10.0	91.7	
$LSD_{5\%}$			1.82	1.51	1.56	0.84	2.56	2.76	0.65	6.84	8.47
F test			8.7***	27.1***	16.6***	4.1**	11.5***	8.0***	19.5***	35.9***	21.4***

** Highly significant at P = 99% probability level

*** Very highly significant at P = 99.9% probability level

[a] controls, DS: durum semolina, WDM: whole durum meal, DSF: defatted soy flour

Significantly lower scores were given to all parameters of WDM and fortified WDM noodles, except the odour test for samples of 100% WDM and for that containing 5% DSF. The panel results showed no significant differences among the experimental samples for all parameters except the odour test. The flavour rating increased with addition of DSF; samples containing 10% or more DSF were rated too strong in flavour. All experimental samples were rated as colored dark brown compared to the controls. The overall scores became lower, when DSF content was increased to 10 or 15%. However, noodles processed from WDM containing 5% DSF was generally preferred over the unfortified WDM noodles and reached 75.6% of the overall acceptability of controls mean.

3. Conclusions

Noodles from WDM or WDM fortified with DSF were not made for replacing traditional pasta products, but for overcoming some domestic problems in Egypt and probably in other developing countries, such as impossibility of semolina purification from native durum wheat because of the absence of the necessary technological background and the protein deficiency in the diet of low income groups.

Incorporation of WDM alone or fortified with DSF in pasta products increased protein content and improved the nutritional properties as a result of the better nutritional balance in soy-cereal blends, increased dough strength and improved cooking properties (except cooking loss) in addition to the reduced production costs compared to the pure DS product. But these products are handicapped by the inferior taste, colour appearance and overall acceptability, as indicated by the taste panel evaluation. However, because pasta is considered to be a nutrient-dense product, pasta made from WDM fortified with 5% DSF could be targeted for low income people in developing countries, as a cheap, high protein, nutritious food.

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ENZYMATIC HYDROLYSIS OF CARROT CELL-WALL POLYSACCHARIDES, IN SITU OR AFTER ISOLATION AS ALCOHOL INSOLUBLE RESIDUE

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Carrot tissue and alcohol-insoluble residue (AIR) from carrot were degraded with cell-wall polysaccharide-degrading enzymes with simulation of maceration (pectinases) and liquefaction (pectinases and cellulases) type treatments. The kinetics of solubilisation and depolymerisation of the polysaccharides, notably pectins and cellulose, the composition of cell-wall material resistant to hydrolysis and the nature of the solubilised polysaccharides were compared. The polysaccharides in AIR were degraded more rapidly than those in the carrot tissue, partially due to the modified structure of cell-walls in the AIR. Notwithstanding, the soluble reaction products, i.e. hairy regions of pectic polysaccharides, were quantitatively similar. In the maceration type treatment, the difference of solubilisation was more significant, owing to the lack of cellulase action.

Keywords: carrot, cell-wall polysaccharides, enzymatic degradation, fruit and vegetable processing

The use of cell-wall polysaccharide-degrading enzymes as processing aids is becoming a normal practice in many sectors of the fruit and vegetable processing industry (ROMBOUTS & PILNIK, 1986). The maceration and liquefaction of plant tissues with enzymes are described by a number of authors (ROMBOUTS & PILNIK, 1978; SREENATH et al., 1986; REISING, 1990). In maceration, the middle lamella consisting mainly of pectin is solubilised by pectinases; a softening and loss of tissue coherence is followed by a release of single cells or cell aggregates. In liquefaction, the cell-walls are degraded by pectinases and cellulases and the cytoplasmic content is released after the breaking of the plasmalemma. Mechanisms of the enzymatic hydrolysis of cell-wall polymers, mainly polysaccharides, have so far been mainly studied on cell-wall preparations (VORAGEN et al., 1980; SREENATH et al., 1984). This material is often an alcohol-insoluble material or a water-insoluble solid which is preserved in a dried form. Modifications and interactions of the cell-wall polymers may occur due to the dehydration effects of alcohol or drying (SELVENDRAN & O'NEILL, 1987), changing the kinetic and the reaction products of enzymatic hydrolysis.

The objective of the present work was to study the enzymatic degradation of cell-wall polysaccharides, in situ. Carrot tissues on the one hand, alcohol-insoluble residue on the other hand were treated with cell-wall polysaccharide-degrading enzymes i.e., pectinases and cellulases. The solubilisation and depolymerisation of pectins and cellulose, and the reaction products were compared. Besides, attempts were made with an enzyme preparation rich in pectinases and depleted of cellulases to follow the hydrolysis of polysaccharides during a maceration type treatment. In this case, maceration was considered as the hydrolysis of pectin by the combination of pectinases and not as the sole effect of endopolygalacturonase.

1. Materials and methods

1.1. Plant material

Carrot roots (*Daucus carota* L.) of the "Nantaise" variety were harvested four months after sowing. Sliced roots were blanched 10 min in boiling water bath before enzymatic treatments.

1.2. Preparation of alcohol-insoluble residues (AIR)

Blanched carrots (200 g) were immersed in boiling aqueous 96% ethanol (1 l), ground with a Waring Blendor for 15 s and blended for 10 min. After filtration through a G3 sintered-glass filter, the alcohol-insoluble material was washed with ethanol until the filtrate was colourless and gave a negative reaction in the phenol-sulphuric acid test (DUBOIS et al., 1956). The residue was washed with acetone and ether, and air-dried. The same procedure was used to obtain AIR from carrots treated with enzymes.

1.3. Enzymes

Two experimental batches of enzymes, E1 and E2 (Table 1) were prepared from commercial mixture, i.e. Maxazym CL 2000, a cellulases-rich preparation (from *Trichoderma viride,* Gist brocades, Seclin, France), Rapidase CPE, a pectinmethylesterase-rich preparation (from *Aspergillus niger,* Gist brocades, Seclin France), SP 249, a pectinases and cellulases-rich preparation (from *Aspergillus aculeatus,* Novo Industri, Bagsvaerd, Denmark), and SP 249 modified by depleting its cellulases (MASSIOT, 1988) by affinity chromatography on cross-linked pectic acid, according to REXOVA-BENKOVA and TIBENSKY (1972).

Table 1

Specific activities and proteins in enzymatic preparations E1 and E2

Activities[a]	E1	E2
Polygalacturonase	2500	2500
Pectinesterase	850	850
Pectin-lyase	25	90
CMCase	0	1100
FPase	0	80
Proteins[b] (mg/essay)	15	30

[a] nkat/essay-250 mg of AIR or 10g of carrot

1.4. Enzymatic treatment of carrot

Degradation of carrot was carried out as follows: 10 g of blanched carrot cut in cubes with edges of 5 mm, were suspended in 50 cm^3 of 0.2 mol l^{-1} sodium acetate buffer pH 4.5 containing 0.02% of sodium azide. Then, 1 cm^3 of enzymatic preparations E1 or E2 (Table 1) was added. The suspension was stirred at 30 °C for 15 to 360 min. At different times of reaction, the mixture was filtered through a G3 sintered-glass filter. The residue (pieces of carrot partially degraded) was washed with water then ground in boiling aqueous 96% ethanol to prepare an alcohol insoluble residue. The filtrate was heated at 90 °C for 5 min by microwaves, concentrated under reduced pressure at 40 °C, treated with 4 volumes of aqueous 95% ethanol and kept overnight at 4 °C. After centrifugation (5000 g, 15 min), the pellet was resuspended in aqueous 80% ethanol and centrifuged as before. The process was repeated three times. The last pellet was dissolved in a minimum volume of water and freeze-dried.

1.5. Enzymatic treatment of AIR

AIR (250 mg) were stirred in 50 cm^3 of 0.1 mol l^{-1} sodium acetate buffer pH 4.5, containing 0.02% of sodium azide. The enzymatic preparation (1 cm^3) E1 or E2 was added and the suspension was stirred at 30 °C. After reaction, the mixture was filtered through a G3 sintered-glass filter; the residue and the filtrate were then treated as above. Each experiment with carrot or with AIR was duplicated and enzymes were omitted for the controls.

1.6. Analytical methods

All the data were on a moisture-free basis. The AIRs were ground (3 min) in a Retsch MM2 mixer mill. The neutral sugar composition of the samples was determined by gas chromatography (capillary column of 30 m × 0.25 mm i.d. with DB 225, 0.15 μm film thickness, J&W Scientific, at 215 °C) using hydrogen as carrier gas, after SEAMAN and co-workers (1954) hydrolysis and derivatisation to alditol acetates (HOEBLER et al., 1989). Myo-inositol was used as internal standard. Galacturonide content was estimated colorimetrically with *m*-hydroxydiphenyl (BLUMENKRANTZ & ASBOE-HANSEN, 1973) after sulphuric acid hydrolysis. In the soluble fractions, galacturonic acid was determined by the automated *m*-hydroxydiphenyl method (THIBAULT, 1979)

1.7. Enzymatic activities

Specific activities were determined as previously (MASSIOT et al., 1989) and proteins were estimated by the LOWRY and co-workers (1951) method using bovine serum albumine as standard.

1.8. High performance gel permeation chromatography

The molecular weight distribution of degradation products was determined by using a HPLC involving a LDC programmable pump equipped with three Bio-Gel TSK columns (each 300 × 7.8 mm) in series (40XL, 30XL and 25XL; Bio-RadLabs) in combination with a TSK XL guard column (40 × 6 mm) at 35 °C. Eluent was 0.4 mol l^{-1} acetic acid; sodium acetate pH 3.6 with a flow rate of 0.8 cm^3 min^{-1}. The eluate was monitored using a thermostated (40 °C) Erma ERC 7512 refractive index detector. The system was calibrated with pectins having molecular weight values in the range 30 000 – 100 000 as determined by viscosimetry (OWEN et al., 1946).

2. Results and discussion

2. 1. Enzymatic degradation of carrot tissues or AIR

The rates of weight loss of cell-wall material originating from AIR or from carrot tissues are shown in Fig. 1. They are expressed in percent of the initial cell-wall material contained in 10 g of fresh carrot, i.e. 250 mg of AIR. The enzyme preparations E1 and E2 (Table 1) contained the same polygalacturonase, pectinesterase and pectin lyase activities; E2 contained cellulases with activities towards carboxy-methylcellulose and filter paper indicating that amorpheous and crystalline cellulose could be hydrolysed with this preparation.

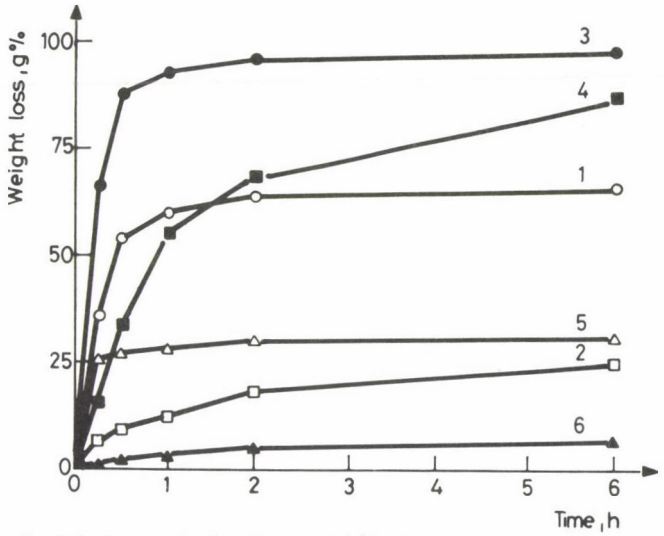

Fig. 1. Kinetics of weight losses of cell-wall material (dry matter g%) after treatment of carrot tissues or AIR with cell-wall polysaccharide-degrading enzymes E1 or E2 (see Table 1) 1: E1/AIR; 2: E1/carrot; 3: E2/AIR; 4: E2/carrot; 5: control/AIR; 6: control/carrot

In the maceration type treatment (E1), the kinetics of solubilisation of the cell-wall material were very different whether the initial substrate was AIR or carrot. E1 solubilised 60 and 66% of the AIR after 1 and 6 h of reaction, respectively, and only 12 and 25% of the carrot cell-wall material after the same times of reaction. In the liquefaction type treatment (E2), 93% of the AIR and 55% of the carrot cell-wall material were solubilised after 1 h. The difference decreased after 6 h (98 and 87%, respectively) suggesting that only the hydrolysis rates were different. After 24 h of reaction, the limits of degradation were the same as those after 6 h.

Controls showed that the weight losses were 7% maximum of the carrot cell-wall material and only 20% of this solubilised fraction were polysaccharides. In contrast, the buffer at pH 4.5 extracted 25–30% of the AIR; half of this water-soluble material was of polysaccharidic nature, indicating that alcohol treatment degraded some cell-wall polysaccharides.

After correction of the control values, the solubilisation of AIR and carrot cell-wall material with E1 was 32 and 9% after 1 h, 35 and 18% after 6 h, respectively; the solubilisation with E2 was 65 and 52% after 1 h, 67 and 80% after 6 h, respectively. Thus, the mixture of pectinases and cellulases was more active on carrot cell-wall material than on AIR.

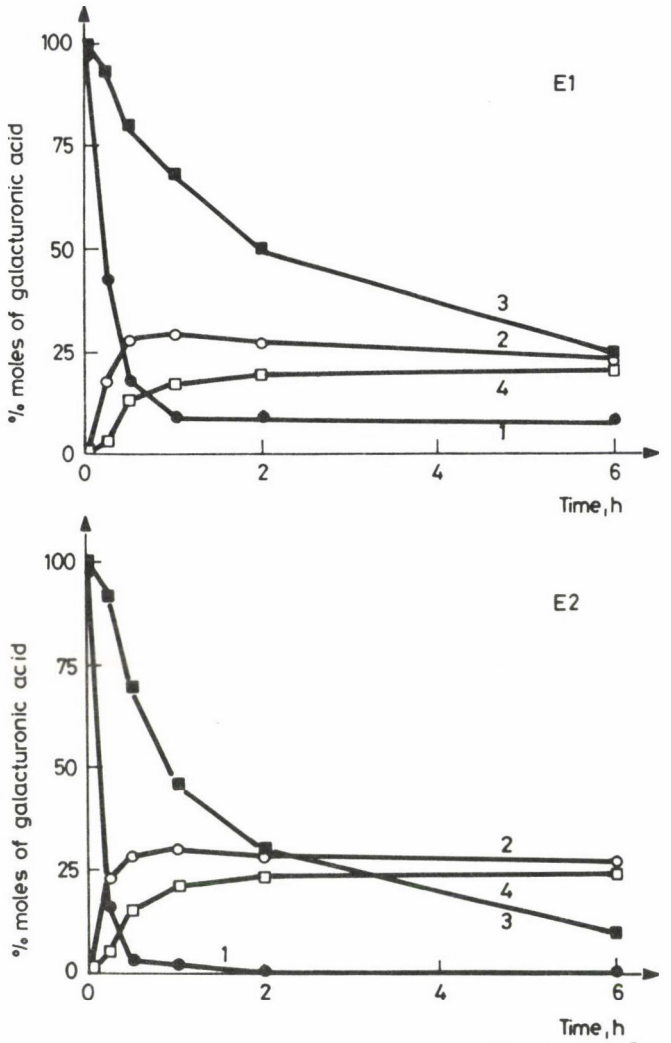

Fig. 2. Content of galacturonic acid (% moles in the initial material) in the water-soluble (precipitating afterwards with ethanol) and insoluble fractions after treatment of carrot tissues or AIR with cell-wall polysaccharide-degrading enzymes E1 or E2 (see Table 1) 1: AIR/insoluble; 2: AIR/soluble; 3: carrot/insoluble; 4: carrot/soluble

2.2. Kinetic study of pectins and cellulose hydrolysis

The solubilisation of pectins (expressed as galacturonic acid) and cellulose (expressed as glucose) are presented in Figs. 2 and 3. The soluble fractions represented the extracted polysaccharides precipitating afterwards with the ethanol.

Degrees of depolymerisation were calculated by the difference between the initial content of sugar and the sum of the contents in alcohol precipitates derived from water soluble and insoluble fractions.

Pectins from AIR were solubilised faster than those from carrot tissue (Fig. 2). The solubilisations with E1 and with E2 were similar. However, the extraction with E1 was slower than with E2 and the extents of solublisation were more important with E2: after 6 h of reaction, 100 and 90% of the galacturonic acid from AIR or from the carrot tissues were solubilised, respectively. The amounts of pectins solubilised with E1 or E2 from AIR arrived at a maximum (ca 30% of galacturonic acid) after 1 h of reaction and then decreased, suggesting, in the soluble phase, pectinase depolymerisation was going on. In contrast, the contents of pectins extracted from carrot increased progressively with the reaction, but in both cases, the final amounts were similar (25%).

The initial rates of solubilisation and depolymerisation (Table 2) showed the rapid hydrolysis of the pectic polysaccharides from AIR. Moreover the depolymerisation was more significant with the presence of cellulases (E2), probably because the hydrolysis of cellulose improved the accessibility of pectins to the pectinases at first. In the treatment of carrot tissues, the rates were ten-fold lower but not significantly different between maceration and liquefaction type treatments, suggesting that, at first, cellulases had no effect on the accessibility of pectins.

Cellulose from AIR or from carrot tissues (Fig. 3) was not hydrolysed with E1; 4% of glucose were recovered in the water-soluble fraction probably from non cellulosic β-glucans. With E2, cellulose from AIR was quickly hydrolysed (2 h) while 70% of the cellulose from carrot tissue were degraded after 6 h of reaction. The rates of solubilisation and depolymerisation (Table 2) were close indicating that cellulose was degraded directly in low molecular weight fragments as previously observed (SREENATH et al., 1984; MASSIOT et al., 1989).

Table 2

Solubilisation and depolymerisation rates of pectins and cellulose[a]

Enzyme		Pectins from AIR	Pectins from Carrot	Cellulose from AIR	Cellulose from Carrot
E1	Solubilisation	9.9[b]	1	0.9[c]	0.5
	Depolymerisation	6.7	0.5	0.7	0.3
E2	Solubilisation	14.6	1.4	12.3	5.9
	Depolymerisation	10.6	0.5	11.2	5.1

[a] determined on the first fifteen minutes

[b] μmoles of galacturonic acid per min

[c] μmoles of glucose per min

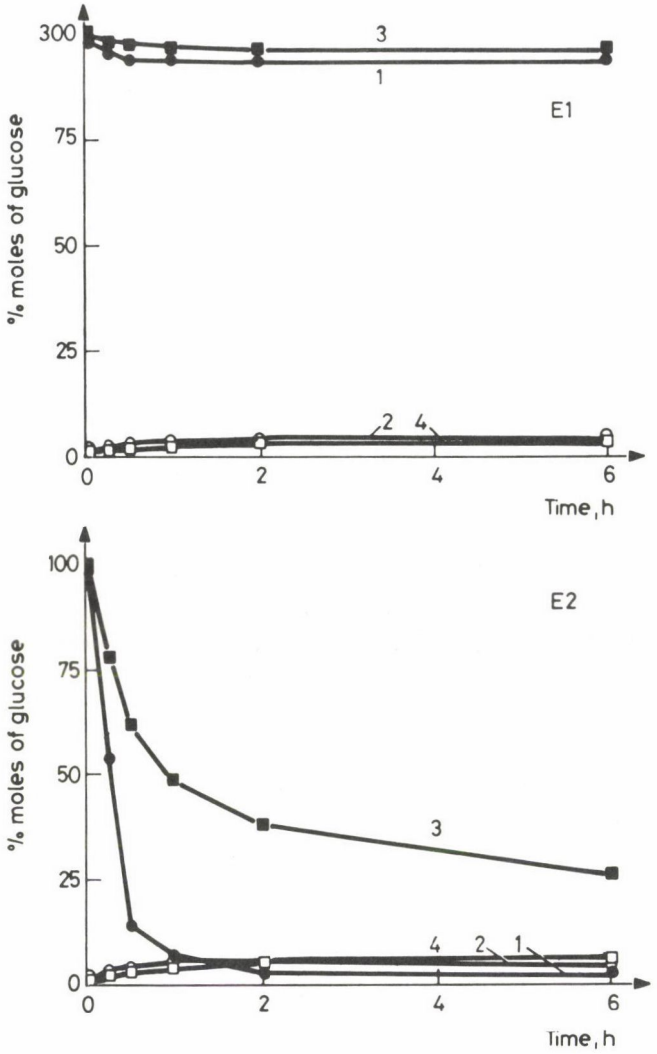

Fig. 3. Content of glucose (% moles in the initial material) in the water-soluble (precipitating afterwards with ethanol) and insoluble fractions after treatment of carrot tissues or AIR with cell-wall polysaccharide-degrading enzymes E1 or E2 (see Table 1). 1: AIR/insoluble; 2: AIR/soluble; 3: carrot/insoluble; 4: carrot/soluble

2.3. Analysis of the cell-wall material resistant to hydrolysis

Data in Table 3 show that the sugar composition of the insoluble materials after enzymatic treatments was different. The carrot cell-wall was composed mainly

of glucose (26.4%), galacturonic acid (24.5%), galactose (10.9%) and arabinose (5.5%), values in agreement with the literature (ASPINALL et al., 1983; VORAGEN et al., 1983; SREENATH et al., 1984; MASSIOT et al., 1988). The AIR degraded with E1 contained mainly cellulose (63.5%) and a low galacturonic content (3.6%), according to the sole action of pectinases. The AIR from carrot treated with E1 represented 75% of the initial cell-wall material but its content of galacturonic acid was 8.9% of the initial content, indicating that pectic substances were preferentially extracted. In contrast, the solubilisation of AIR polysaccharides with E2 was almost complete, in agreement with a liquefaction type treatment. Only, a fraction (8.4%) originating from xyloglucans and/or cellulose resisted to the action of cellulases. The AIR from carrot treated with E2 accounted to 13% of the initial content and contained 48.6% of glucose, showing the uncomplete action of cellulases on the carrot tissues.

Table 3

Sugar composition of AIR from carrot treated and untreated with enzymes (after 6 h treatment)

	AIR	AIR treated with E1	AIR treated with E2	AIR from carrot treated with E1	AIR from carrot treated with E2
Recovery(%)	100	34	2	75	13
Galacturonic acid (g per 100 g)	24.5	3.6	0	6.2	8.5
Rhamnose	1.8	0.5	0.6	0.6	0.5
Arabinose	5.5	1.3	0	2.7	3.1
Xylose	1.1	2.7	5.1	2.1	3
Mannose	1.5	2.5	0	1.7	0.2
Galactose	10.9	1.9	0.2	3.2	5.2
Glucose	26.4	63.5	2.5	35	48.6
Total	71.7	76	8.4	51.5	69.1

2.4. Characterisation of soluble polysaccharides

Table 4 shows the sugar composition of the polysaccharides solubilised after 15 min and 6 h of reaction. The polysaccharides extracted from AIR or carrot showed a similar composition. These fractions, mainly composed of galacturonic acid, galactose, arabinose and rhamnose, were probably the hairy regions of pectic polysaccharides which were resistant to enzymes. The hydrolysis of side chains of pectic polysaccharides was more extended with E2 because the soluble fractions contained less galactose and arabinose and more galacturonic acid and rhamnose than those extracted with E1. In all cases, the molar ratio of galacturonic acid: rhamnose decreased with the time of reaction, indicating that, in the soluble phase, the fragmentation of the homogalacturonic zones was going on.

Table 4

Sugar composition of soluble fractions released from AIR or carrot tissues with cell-wall polysaccharide-degrading enzymes E1 and E2
(see Table 1)

Enzyme	Substrate	Time of reaction (h)	Yield (%g of cell-wall material)	Sugar composition[a] (% moles)						
				Gal A	Rha	Ara	Xyl	Man	Gal	Glc
E1	AIR	0.25	8.5	36.5	5.0	16.3	0.2	1.7	33.6	6.7
		6	11.1	23.9	6.4	23.7	0.4	1.6	40.3	3.7
	Carrot	0.25	2.1	34.9	3.0	21.4	0.3	3.8	33.8	2.8
		6	7.0	36.7	3.4	23.5	0.2	1.1	31.7	3.4
E2	AIR	0.25	8.2	54.1	7.8	8.3	0.4	4.9	18.4	6.1
		6	9.1	61.4	13.5	7.2	0.6	5	6.4	5.9
	Carrot	0.25	2.4	42.6	8.8	9.5	0.6	13	15.8	9.7
		6	7.5	47.6	13.6	9.8	1.1	8.3	15.5	4.1

[a]Gal A, Rha, Ara, Xyl, Man, Gal and Glc denote Galacturonic acid, Rhamnose, Arabinose, Xylose, Mannose, Galactose and Glucose, respectively

The molecular weight and the homogeneity of soluble polysaccharides were determined by using high performance GPC (Figs. 4 and 5). In the maceration type treatment (E1), the fraction extracted from AIR contained one main peak (22.8 min, mol. wt ca 50 000) after 6 h of reaction, whereas three distinct populations were solubilised from the carrot. In the liquefaction type treatment (E2), one homogeneous population (27.4 min, mol. wt ca 10 000) was present after 6 h, both from AIR and from carrot. This observation was in agreement with data of Table 4 which show that hydrolysis of the pectic polysaccharides was more extented with E2. E1 and E2 contained probably different enzymatic activities as arabinase or galactanase (not determined).

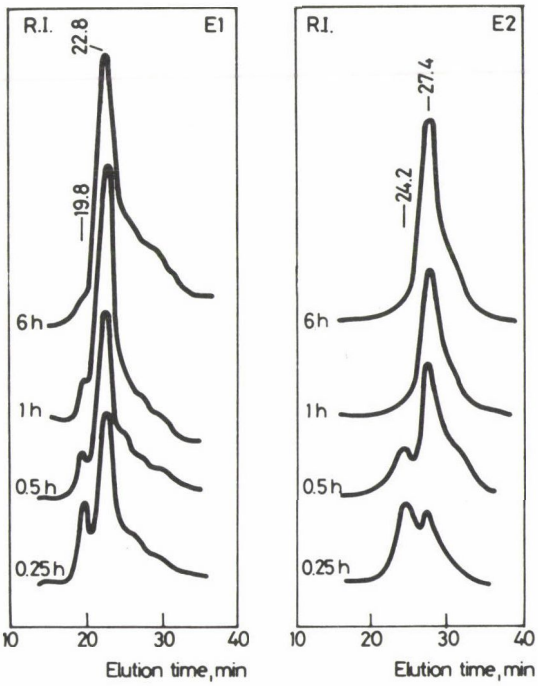

Fig. 4. Analysis with high performance gel permeation chromatography of the molecular weight distribution of water soluble fractions (precipitating afterwards with ethanol) after treatment of carrot tissues AIR with E1 or E2 (see Table 1)

3. Conclusions

The foregoing results show that cell-wall polysaccharides isolated in AIR were solubilised and depolymerised with enzymes more rapidly than those in native form in the carrot tissues. However, in the liquefaction type treatment with the simultaneous action of pectinases and cellulases, the limits of degradation were similar. These observations might be explained, partially, by the spontaneous solubilisation of polysaccharides (ca 20%) from AIR in the sodium acetate buffer pH 4.5 at 30 °C while only a little fraction (ca 2%) was extracted from carrot. The way of preparation of AIR modified the behavior of cell-wall polysaccharides towards enzymes. The precipitation in alcohol and the mechanical effects of grinding probably altered the structure of the cell-wall (SELVENDRAN & O'NEILL, 1987) and some polysaccharides as pectins were easily solubilised. While the first phase of the reaction, cellulose from carrot tissue was not accessible for cellulases, cellulose from AIR was quickly hydrolysed. The difference between the two rates was likely related

with the diffusion of enzymes to their substrates as well as the diffusion of the products of reaction which was probably easier in the case of AIR.

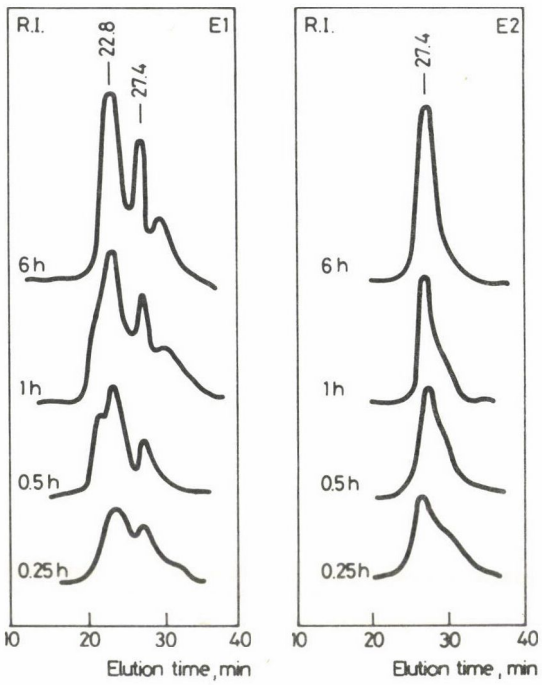

Fig. 5. Analysis with high performance gel permeation chromatography of the molecular weight distribution of water soluble fractions (precipitating afterwards with ethanol) after treatment of carrot tissues with E1 or E2 (see Table 1)

In the maceration type treatment, the amounts of pectins solubilised from AIR were more important than those solubilised from carrot. In this case, the cellulose was not hydrolysed and the pectic polysaccharides from the primary cell-wall were probably less accessible in the carrot tissues (SCREENATH & RADOLA, 1986).

In all these enzymatic degradations, the soluble polysaccharides resistant to an extended depolymerisation represented ca 10% of the cell-wall material. In the soluble phase, the fragmentation of pectins with polygalacturonases and pectin-lyases were probably similar and led to the same products. These were the hairy region of pectic polysaccharides with side chains of arabinans and galactans, as previously observed on carrot (MASSIOT et al., 1989) or on apple (SCHOLS et al., 1990).

The enzymatic hydrolysis of cell-wall polysaccharides isolated on AIR form led to similar products than the degradation of cell-wall, in situ, in the plant tissues. Nevertheless, the initial mechanisms, as diffusion of enzymes and accessibility of substrates were different in the first phase of reaction. The extrapolation of results

obtained on AIR studies to cell-walls of plant tissues should be taken into account as a latency period due to the accessibility of polysaccharides. Our current studies on apple cell-wall polysaccharides indicate similar observations. Thus, AIR, recognized as important starting material for cell-wall analysis is not fully representative of the behavior of natural cell-wall polysaccharides towards enzymes.

*

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PURIFICATION OF HORSERADISH PEROXIDASE BY THREE-PHASE PARTITIONING (TPP)

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Deep-frozen horseradish gratings were extracted with a solution composed of 0.1 mol l^{-1} K_2HPO_4/0.1% Tween 80. Crude horseradish juice was first fractioned by three-phase partitioning (TPP). Upon increasing the pH of the solution saturated to 50% to 9.0 and its saturation to 80% $(NH_4)_2SO_4$, peroxidase of RZ = 0.3 was precipitated as third phase. By chromatography of the dissolved and dialyzed enzyme solution over CM-cellulose, high purity peroxidase (RZ > 2) was obtained.

Keywords: horseradish peroxidase, three-phase partitioning, CM-cellulose chromatography

From the peroxidases widespread in nature, purified horseradish peroxidase is used in large amounts for histochemical, clinical and analytical purposes.

Several procedures are known for purifying the enzyme (BRATTAIN et al., 1976; CHIA-HSIU et al., 1980; BEHN et al., 1987; CASL & MALNAR, 1986; LASCU et al., 1986) in which fractionation of high-purity peroxidase is achieved either by increasing the steps of purification or by applying specifically interacting substances (e.g., Sepharose CL-6B, a monoclonal antibody or concanavalin A).

Three-phase partitioning, which has become known in the recent past, can be applied, in general, for purifying protein or enzyme solutions (ODEGAARD et al., 1984; LOVRIEN et al., 1987).

In the following an account will be given of a combination of three-phase partitioning and an earlier developed procedure for peroxidase fractionation.

1. Materials and methods

1.1. Horseradish (Armoracia lapathifolia)

Ripe horseradish roots were obtained from some farms. The washed roots were stored at -20°C.

1.2. Preparation of horseradish peroxidase

1.2.1. Conminution and extraction. The roots cooled to -20°C were comminuted in a household machine (KM-8, Aka Electric, perforated steel plate for vegetable grating). Horseradish gratings were extracted, at a ratio of 1:3 (w/v), with a 0.1 mol l^{-1} K_2HPO_4 solution containing 0.1% Tween 80 (SHANNON et al., 1986; SCOPES, 1982). Extraction was performed, in general, at 0-4 °C, in some cases at higher temperatures. The crude enzyme solution was separated from the fibers by filtration.

1.3. Three-phase partitioning

Tert. butanol, which is water-miscible in any proportion, forms a separate phase on a $(NH_4)_2SO_4$ solution saturated to 20-40%. If the aqueous phase contains proteins, part of them forms, after vigorous shaking, a so-called midlayer between the lower aqueous and the upper tert. butanol phase, and can be separated (ODEGAARD et al., 1984; LOVRIEN et al., 1987; PIKE & DENNISON, 1989).

In the given purification experiment $(NH_4)_2SO_4$ saturated to 50% was added to crude, filtered horseradish juice, the precipitate obtained was centrifuged (20 min, 2500 g), tert. butanol was added to the supernatant in an amount of 1:5 of its volume and then this mixture was treated for 1 min in an Ultra Turrax device (9500 r.p.m., S 25 N Schaft). After centrifuging at low speed (1000 r.p.m.), the pH of the combined aqueous phases was adjusted to 9.0 with NH_4OH or 40% NaOH, then $(NH_4)_2SO_4$ was added to 80% saturation. Then 1:50 volume of tert. butanol was added under stirring and the solution was allowed to stand overnight. The protein precipitate formed on the surface of the liquid was separated in a separating funnel from the liquid phases.

1.4. Purification by column chromatography

The brown precipitate resulting from three-phase partitioning was dissolved in a minimum volume of 0.05 mol l^{-1} Tris (pH 7.0), then dialyzed against 0.005 mol l^{-1} sodium acetate (pH 4.4).

Pre-swelled CM-cellulose (fiber, 0.6-0.8 meq/g, Reanal, Hungary) was filled in a plastic column of 100 cm^3 volume (4.5 × 7 cm). The column was equilibrated with 0.005 mol l^{-1} sodium acetate (pH 4.4) (SHANNON et al., 1966). Equilibration was checked with a conductometer.

The dialyzed protein solution was applied to the CM-cellulose column, the protein fraction bound to the column was washed with 0.005 mol l^{-1} acetate buffer (pH 4.4), then peroxidase was eluted with 0.1 mol l^{-1} acetate buffer (pH 4.4). Changes in the protein content of the eluate were followed with a flow-cuvette UV spectrophotometer (Spektromom 195 D).

1.5. Measurement of peroxidase activity

Peroxidase activity was measured according to the Sigma method (ANON, 1983). One Sigma activity unit = 1.0 mg purpurogallin formed from pyrogallol in 20 sec at pH 6.0 and 20°C.

1.6. Protein determination

The protein content of the samples obtained in the course of fractionation was measured by the biuret method (SCHLEIF & WENSINK, 1981). Sample of 300 μl were added to 3 cm^3 biuret reagent and A_{550} was measured after 20 min against reagent plus buffer; 0.06 A_{550} corresponds to 0.2 mg protein.

2. Results and discussion

The combined application of two known procedures (SHANNON et al., 1966; ODEGAARD et al., 1984) allowed to obtain high purity horseradish peroxidase in relatively few purification steps.

Extraction of peroxidase and other proteins was performed by comminution of horseradish at -20°C and subsequent extraction of the gratings. The dependence of the extractable enzyme activity on the duration and temperature of extraction is shown in Fig. 1. Peroxidase gets quickly dissolved, the activity measured on mixing the gratings with the extracting solution amounted to 88% of maximum extracted activity.

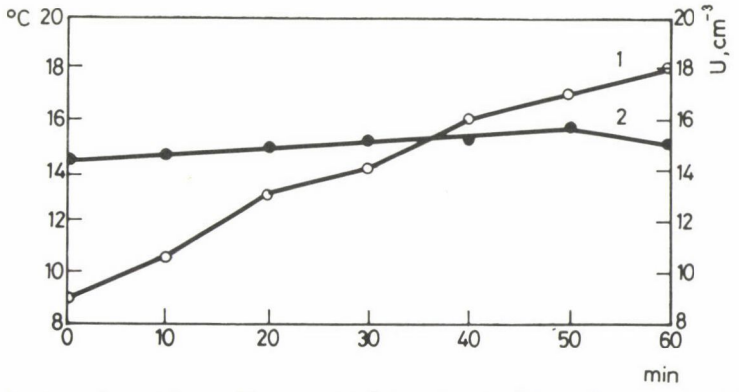

Fig. 1. Activity dependence of peroxidase extracted from horseradish gratings on extraction time and temperature. 150 g horseradish gratings were stirred for 60 min with 450 cm^3 solution composed of 0.1 mol l^{-1} K_2HPO_4/0.1% Tween 80. 1: change in temperature during extraction, 2: activity of dissolved peroxidase

Three-phase partitioning, with tert. butanol and a 1:1 mixture of tert. butanol and *n*-butanol, respectively, of the supernatant saturated to 50% with ammonium sulfate is shown in Fig. 2. *n*-Butanol, which mixes with water to a much lesser extent, was applied as a possible alternative of phase separation by centrifuging. The two kinds of organic phases caused practically identical changes in the A_{280} values (4.0 and 3.6, respectively) which were modified but to a slight extent by the second partition (with a 9:1 mixture). The organic phase containing also *n*-butanol yields a quicker and sharper separation from the aqueous phase (Fig. 3), and the decrease in protein content is but slightly inferior as compared to partitioning with pure tert.

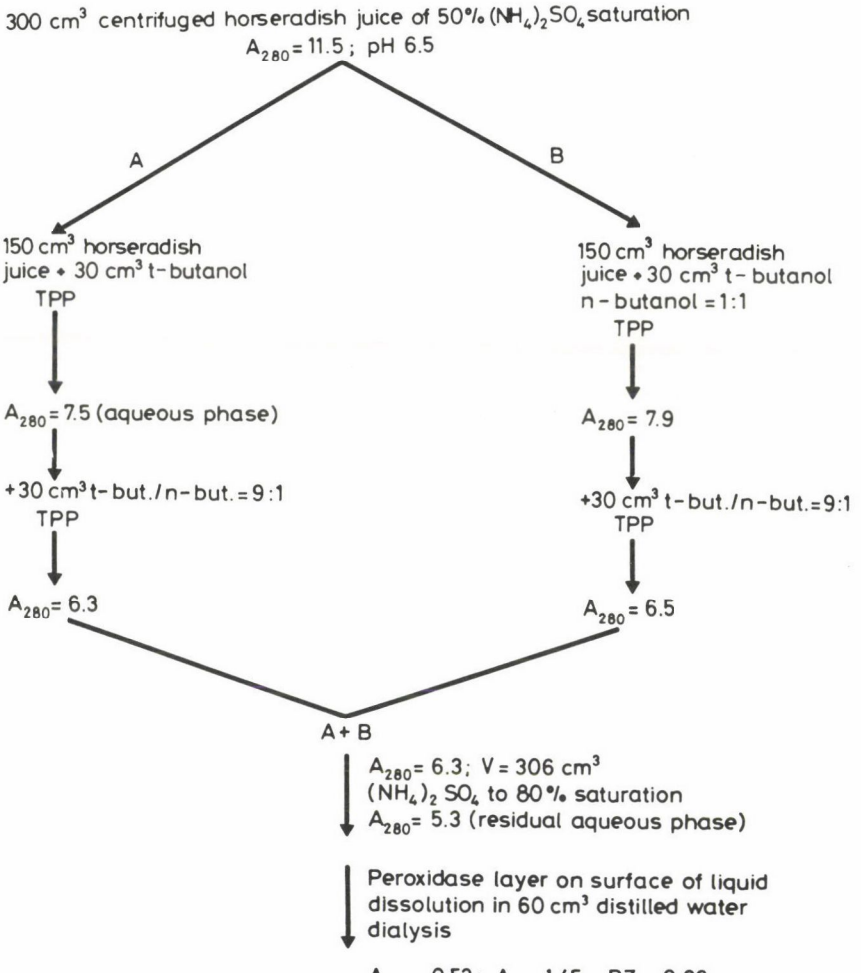

Fig. 2. Three-phase partitioning with *t*-butanol and a mixture of *t*-butanol and *n*-butanol, respectively. Changes in protein content were followed by absorbance measurements at 280 nm. $RZ = A_{403}/A_{275}$

butanol. By increasing ammonium sulfate saturation of the combined aqueous phases to 80% and adding ter. butanol in an amount 1:50 volume of the aqueous phase, crude peroxidase is obtained on the surface of the liquid. RZ (Reinheitszahl) of the separated, dissolved and dialyzed enzyme was, in this experiment, 0.36.

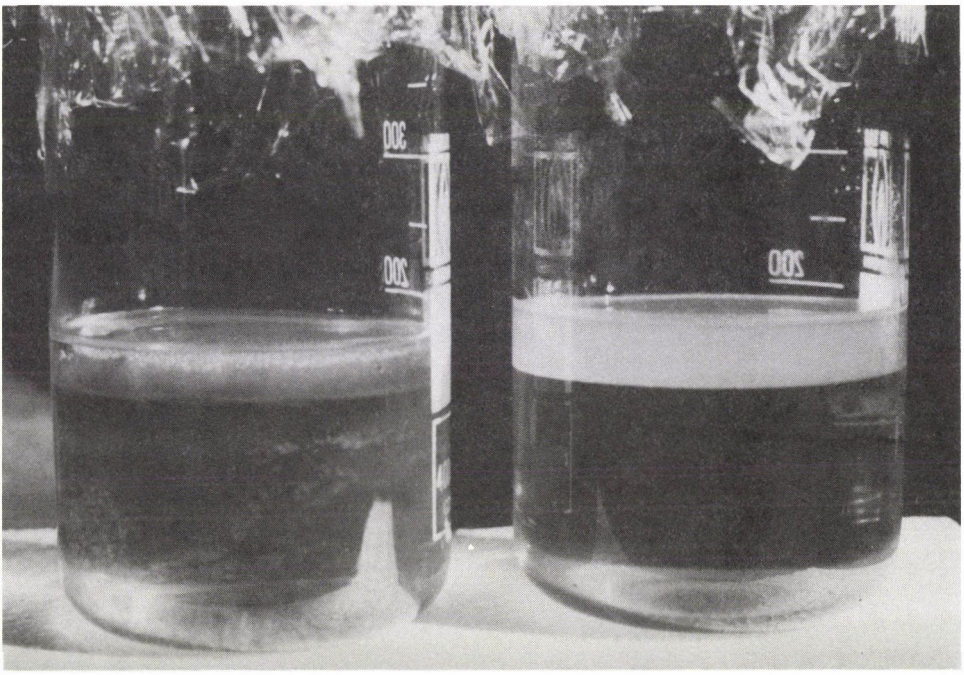

Fig. 3. Effect of *n*-butanol on three-phase partitioning. On the left hand side of the Figure TPP I, carried out with tert.butanol according to section A of Fig. 2, can be seen; the right hand side shows TPP carried out with the mixture tert.butanol and *n*-butanol according to section B. The protein disk is formed by mainly inactive accompanying proteins. Non-centrifuged samples, separation occurred spontaneously

On the basis of the results of the laboratory experiments the purification procedure was reproduced on a pilot laboratory scale. Eight kg horseradish gratings were treated with 20.2 dm^3 extracting solution, using 10 kg ammonium sulfate and 3.7 dm^3 tert. butanol for three-phase partitioning. The purification procedure is shown in Table 1. A modification of the previous experiment (Fig. 2) consisted in the fact that only one TPP step was performed with *t*-butanol at 50% saturation, whereafter the pH of the solution was adjusted to 9.0. At the given ammonium sulfate concentration (50%) and the pH shifted to about the isolectric point – under given conditions the so-called neutral peroxidase isoenzymes (AIBARA et al., 1982) can be found in the aqueous phase – the isoenzymes in solution do not precipitate (PIKE & DENNISON, 1989), at the same time they practically completely sediment out of the aqueous phase when the ammonium sulfate concentration is increased to 80%.

Table 1

Purification of horseradish peroxidase by three-phase partitioning and ion-exchange chromatography

Sample	Volume (cm^3)	Activity (U cm^{-3})	Total activity (U)	Protein (mg cm^{-3})	Total protein (mg)	Specific activity (U mg^{-1})	Yield (%)	Purification
Crude horseradish juice	20200	19.1	385 820	7.0	141 400	2.7	100	–
Supernatant of 50% $(NH_4)_2SO_4$ Saturation	20400	12.4	252 960	0.8	16 320	15.5	65.6	5.7
TPP I (50%)	20300	10.9	221 270	0.5	10 150	21.8	57.4	8.1
TPP II (80%, pH 9.0) surface protein layer + TRIS (pH 7.0)	2000	57.0	114 000	0.9	1 800	63.8	29.5[a]	23.6
CM-cellusose chromatography[b] 0.1 mol acetate buffer (pH 4.4) fraction (335 cm^3)	94	–	–	0.47	44.5	250.0	17.1	92.6

[a]Average yield of five separate experiments (TPP II) : **0.3 ± 0.04, scale 1-5 dm^3**
Reinheitszahl (RZ) of the intermediates obtained with TPP II (4 experiments): 0.3 ± 0.4, scale 1-5 dm^3
[b]335 cm^3 of the 2000 cm^3 solution obtained after TPP II were applied to the CM-cellulose column

The RZ = 0.3 enzyme solution obtained by dialysis of the surface protein layer was adjusted to 0.005 mol l^{-1} acetate buffer concentration (pH 4.4); when chromatographed on low ion-exchange capacity CM-cellulose, this yielded an enzyme of RZ = 2.0 – 2.5.

3. Summary

By the extraction and subsequent three-phase partitioning of horseradish roots comminuted in deepfrozen state, a crude enzyme solution was obtained which allowed to obtain, by single stepwise elution from CM-cellulose, peroxidase of high purity.

After pulverization of the -20°C horseradish, an important step of purification is three-phase partitioning carried out at 50% (I) or 80% (II) ammonium sulfate saturation. While in the first case the midlayer is formed by a great amount of inactive protein and a small amount of peroxidase (about 7%), after partitioning II practically the total amount of active peroxidase gets to the surface of the aqueous phase, in the form of a brown disk. By processing peroxidases of different origin, it was established that the peroxidase/other protein ratios in this brown protein disk and the protein solution that can be obtained from it by dialysis/ultrafiltration are

very similar, and that the other proteins can be separated from the enzyme by simple CM-cellulose chromatography.

The application of the mixture tert.butanol / *n*-butanol is justified when a great volume of solution has to be dealt with, and there is no possibility of centrifuging. The discoloured organic solvent can be purified and recovered, respectively, by distillation, while the residual solution of 80% saturation may be applied as liquid fertilizer.

Figure 4 shows the NIR spectra of analytical grade and fertilizer grade ammonium sulfate, respectively. Differences can be observed in the spectrum section above 2000 nm. It is the result of an independent experiment that fertilizer grade $(NH_4)_2SO_4$ can be applied without any limitation for horseradish peroxidase purification as three-phase partitioning eliminates the impurities present in the fertilizer.

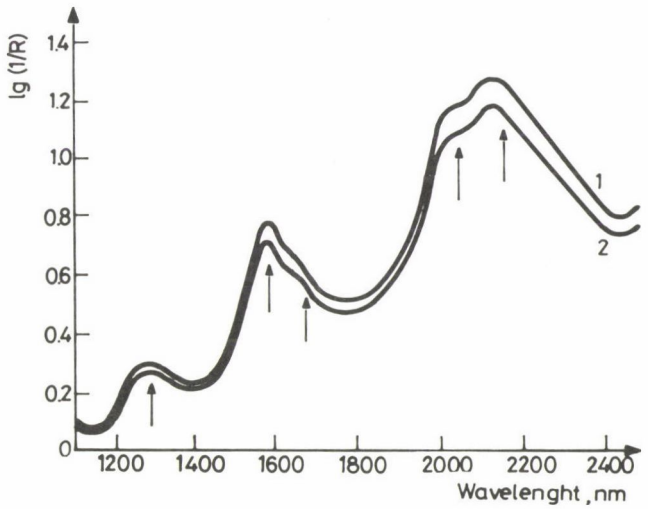

Fig. 4. NIR spectra of analytical grade (1) and technical grade (2) ammonium sulfate

*

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EFFECT OF BLANCHING AND RIPENESS ON GREEN PEA PEROXIDASE ACTIVITY

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Effectiveness of blanching is rated on the basis of residual peroxidase (POD) activity. The *o*-phenylene diamine method according to Winter was applied as activity assay throughout our research work. Experimental samples were manufactured under plant conditions. Modernisation of blanching technology was aimed at in order to achieve improved quality. The influence of the method of blanching (steam, water and individual quick-blanching) and of the blanching parameters (blanching temperature, blanching time) upon peroxidase activity of green peas to be quick-frozen, were investigated. Samples were stored for 12 months at −20 °C. Changes in POD activity were checked also during storage time.

It was assumed that POD activity was dependent on the ripeness of green peas, i.e., enzyme activity was suited to indicate picking ripeness with progressing maturity. Green peas of given varieties were investigated for establishing the course of ripening, and relationships were sought for between the properties characterizing ripening (Finometer degree, size of individual peas, mass of thousand peas, moisture content) and peroxidase activity.

The results of our investigations allowed to conclude that blanching as applied in industrial practice is exaggerated. Inactivation of the enzyme peroxidase occurs in the temperature range 82–85 °C. This is the important section, from the aspect of blanching, and it is not worthwhile and not economic to surpass these temperatures. The knowledge of POD activity is an important measure of value in the technological process. It is not sufficient to know only the tenderness of the raw material as, according to varieties, different POD activity values belong to the same value of tenderness. Time and temperature of blanching must be chosen to yield a residual enzyme activity of 5–6%. Green peas are blanched without sorting according to size. Therefore achievement of suitably low POD activities even in the peas of the greatest diameters have to be considered when setting blanching parameters.

Reactivation of green pea POD during frozen storage is minimal.

Keywords: blanching, ripeness of green pea, peroxidase activity, heat treatment

One of the objectives of blanching is to inactivate the enzyme system of the commodity by applying high temperatures and thus to slow down quality deterioration during frozen storage. Moreover, blanching has a number of other advantages including excellent colour stability, improvement of texture, decrease in viable counts, elimination of undesirable substances, etc. However, the operation has also disadvantages, such as decrease in nutritive value and water pollution. Some vegetables keep their quality in the frozen state even without previous blanching (KOZLOWSKI, 1980). According to the investigations of CARLES (1984) some frozen raw materials to be further processed can be stored at −30 °C up to 3 months, without noteworthy changes in quality. With mushrooms, celery and asparagus, which

cannot be frozen without blanching, STEINBUCH (1979, 1980) successfully applied vacuum packaging instead.

Most vegetables are, however, frozen after blanching. Research is aimed at diminishing the adverse effect of blanching upon vegetable quality. Some authors strive to improve milder procedures (PHILIPPON, 1984; POULSEN, 1986) while others wish to modernize the equipment (STEINBUCH, 1983; PHILIPPON, 1984). TOGEBY and co-workers (1986) aimed at reducing energy consumption of the process by modeling mass losses and enzyme inactivation in industrial processes.

PHILIPPON and ROUET-MAYER (1984) investigated the effects of heat treatment on the enzyme system peroxidase – catalase – lipoxigenase. The efficiency of heat treatments is, in general, controlled by measuring the activities of peroxidases or catalases. The higher heat stability of peroxidases is dependent, in the first place, on their location in the plant tissues. Catalase can be found in the cytoplasm, while peroxidase is mainly cell-bound.

Enzyme activities vary considerably also with vegetable varieties. PIZZOCARO and co-workers (1988) found, in the families of Cruciferae and Leguminosae, peroxidase activities to be prevailing, while in the family of Solanaceae the activities of the enzyme lipoxigenase, were highest. Opinions are divergent as to the control of which enzyme might be suitable to indicate the extent of blanching. WILLIAMS and LIM (1986) carried out the comparable evaluation of a number of enzymes in plant raw material. Several methods have been developed for controlling activity (ADAMS, 1978). For on-line control the simple tests proved suitable. These apply previously an indicator dye onto paper strips or disks which gets discoloured when brought in touch with a material containing peroxidase.

In domestic practice the control of peroxidase enzyme activity in raw materials for the deep-freezing industry has come into general use. BARTUCZ-KOVÁCS (1979) advised a residual enzyme activity below 6% for green peas. Peroxidase activity shows considerable variations not only among various kinds of vegetables but even within a given kind of vegetable. The stability of the enzyme varies according to the ratio of the heat stable and heat labile enzyme fractions. The extent of regenerability has been studied by MIHÁLYI and VÁMOS-VIGYÁZÓ (1976).

In the course of our work aimed at updating blanching technology, we strived to achieve improved product quality by decreasing temperature and time of blanching. Further it was investigated how the ripeness of green peas affected peroxidase enzyme activity.

1. Materials and methods

1.1. Blanching conditions

Samples to be analyzed were produced in plant equipment on the production lines of the Békéscsaba Deep-Freezing Company (Békéscsabai Hütõipari Vállalat). After blanching, the samples were frozen in a fluidization freezer and stored for 12 months at −20 °C.

Blanching was carried out in a bucket blancher equipped with a level-height indicator. The equipment could be controlled automatically taking into account the mass flow.

During the experiment, blanching was performed in the blanching equipment made by Cabinplant International, Denmark, both in water and in steam. This equipment is provided with adjustable band velocity, is of the IQB system and energy saving. Its inset heat exchanger provides for advantageous water consumption. Cooling is solved by a combination of dipping in cold water and applying a storing air flow.

1.1.1. Symbols of the samples produced for investigating the effect of the blanching procedure on peroxidase activity

N: green peas frozen without blanching

W: green peas water-blanched in the bucket blancher for 4 minutes at 93 °C and 85 °C, respectively (W93, W85)

C: green peas water-blanched in the Cabinplant equipment at 95 °C, for 1 and 2 min, respectively (C1, C2)

S: green peas steam-blanched in the Cabinplant equipment at 100 °C in direct vapour for 1 and 2 min, respectively (S1, S2)

1.1.2. Effect of blanching time and temperature on peroxidase enzyme activity. In the industry water blanching is traditional, therefore it was investigated how time and temperature of this kind of heat treatment affect enzyme activity.

Blanching temperature was adjusted to 86 and 82 °C, respectively, while blanching time was 3'50" in both cases. When heat treatment was performed at 90 °C, blanching times were 2'15" and 3'10". The industrial sample was used as control. This was blanched, according to the quality of the raw material, at 90 °C for 3'50".

1.1.3. Effect of pea diameter on peroxidase activity. Green peas blanched according to the procedures described in paragraph 1.1.1. were divided in two groups according to size. One group contained the peas of individual diameters of 6–7 mm, the other those of diameters exceeding 9 mm.

In another experiment peroxidase activity was determined in a sample produced in the industry, was frozen subsequent to blanching and sorted according to pea size to groups of 6 – 8, 9 – 10 and > 10 mm for enzyme assays.

1.2. Quality of the raw material

1.2.1. In the blanching experiments peas grown in the own production area of the factory, were used. The raw material was qualified according to tenderness. An IMC Tenderometer was used as measuring instrument.

The tenderness of the samples prepared according to 1.1.1. was 120 °T ± 7.5, while tenderness of those prepared according to 1.1.2. was 92 °T ± 10.4.

1.2.2. For investigating the course of ripening 6 varieties were used. These were grown, by the Institute for Vegetable Growth of the University of Horticulture and Food Industry, on an experimental parcel, and were partly qualified by the state institutions as suitable for commercial growth, and partly designated for qualification. Sampling was performed weekly, on approaching the tenderness value of 100 °T.

The marrowfat pea varieties investigated were:

Ave
Booker seed
Margit
Maxigolt
Polar
Ujmajori középkésõi (Middle-late from Ujmajor).

1.3. Methods of investigation

The peroxidase enzyme activity assay was carried out by the ortho-phenylene diamine method of WINTER (1969). Ripeness was tested by measuring tenderness with the instrument Finometer (TÖRÖK & KISZEL, 1969).

Moisture was determined by drying at 105 °C till reaching constant mass.

The mass of 1000 peas was calculated from measurements of mass of 100 peas with an accuracy of 0.01 g. Pea diameter: mean of the longitudinal diameters of 50 peas.

2. Results

2.1. Effect of the blanching method on peroxidase enzyme activity

The results are shown in Table 1, mathematical-statistical evaluation of experimental data is presented in Table 2.

Table 1

Effect of the blanching method on peroxidase activity in green peas

Pea diameter (mm)	Blanching method	Duration (months) 0 $\bar{x}$	0 ±s	2 $\bar{x}$	2 ±s	6 $\bar{x}$	6 ±s	12 $\bar{x}$	12 ±s
		Residual POD activity (U g^{-1})							
above 9	N	29509	2244	19210	730	19885	1284	19905	101
	S1	14129	799	8434	327	6956	144	4716	74
	S2	220	19	72	11	7	3	20	2
	C1	193	18	149	28	106	8	127	1
	C2	19	4	5	1	0	0	5	0
	W85	495	27	503	39	580	36	535	1
	W93	2	1	102	8	2	0	3	0
6-9	N	27782	490	23905	3944	22624	302	17431	196
	S1	14129	799	6846	484	6950	236	8341	85
	S2	404	5	254	23	46	6	83	1
	C1	251	43	406	10	98	5	69	1
	C2	110	13	87	5	84	4	63	1

Frozen storage was carried out at −20 °C temperature
Number of parallel measurements: n = 3
For symbols used for blanching methods see para 1.1.1.

Investigations were carried out immediately after blanching and freezing as well as after storage periods of 2, 6 and 12 months. Peroxidase enzyme activity in peas ranges from 27 000 to 30 000 U g^{-1}. This represents a medium value among vegetables, and applying blanching in steam for 1 min (S1) reduces enzyme activity to about half its original value. This residual enzyme activity is still high, thus this blanching method is unsuitable. As the POD activity values of unblanched (N) peas and of those blanched in steam for 1 min (S1) belong to different orders of magnitude, mathematical evaluation was performed without these two samples, in order to be able to establish the differences between the rest of treatments.

All other blanching methods reduced the residual enzyme activity compared to that of the untreated green peas below 2 percents. The other of the treatments after blanching was – from the point of view of enzyme inactivation – as follows:

pea size exceeding Ø 9 mm

1. W93 and C2
2. C1 and S2
3. W85
4. S1
5. N

pea size of Ø 6mm

1. C2
2. C1
3. S2
4. S1
5. N

Table 2

Results of two-way variance analysis for the investigation of the effect of blanching method

Pea diameter (mm)	Blanching method	Duration of frozen storage (months) 0	2	6	12	Independently from storage period
above 9	N	a	a	a	a	a[a]
	S1	b	b	b	b	b
	S2	d	e	e	e	e
	C1	d	d	d	d	d
	C2	e	f	e	f	f
	W85	c	c	c	c	c
	W93	e	de	e	f	f
6-9	N1	a	a	a	a	a
	S1	b	b	b	b	b
	S2	c	d	e	c	c
	C1	d	c	c	d	c
	C2	e	e	d	e	d

Effect of treatment related to each other at 99% probability level in function of the blanching method
[a]significant at 95% probability level

Effect of treatment as a function of storage time (SD 99%)

Pea diameter (mm)	Storage time (months)	Blanching method S1	S2	C1	C2	W85[b]	W93
above 9	0	a	a	a	a	a	a
	2	b	b	b	b	a	b
	6	c	c	c	b	b	a
	12	d	c	bc	b	ab	a
6-9	0	a	a	b	a		
	2	c	b	a	b		
	6	c	d	c	b		
	12	b	c	c	c		

[b]at probability level of 93%

For the two kinds of size diameters, the same order has been observed from the point of view of the blanching method. There was no significant difference found between the enzyme activity of green peas water blanched at 93 °C temperature (W93) and blanched for 2 min in water in the Cabinplant. Here the blanching temperature was 95 °C. Both blanched states point to an overcooking. POD activity values of the samples were minimal. It can be observed, that the water blanching

results in a better inactivation than steam, considering identical conditions of blanching time and at about similar blanching temperatures.

The initially measured differences in enzyme activity decreased during strorage. The effect of storage time for the greater pea sizes could be proved significantly. In the first phase of the storage at −20 °C temperature, enzyme activity measurements carried out more frequently in shorter storage periods would give more precise answers on the behaviour of peroxidase enzyme. During frozen storage, the reactivation of peroxidase enzyme is not to be reckoned with following the treatments applied in the investigations. During storage a further reduction in enzyme activity has been observed that could be seen from the results of POD assays made in the 2nd and 6th months. In the second half of storage time, these values got stabilized, according to mathematical statistical evaluations, no significant differences have been caused by the storage period.

For pea diameters of 6−9 mm, unambigously significant changes were detected during storage time. During frozen storage, a decreasing peroxidase enzyme activity was measured in case of sample C2 with the least enzyme activity. At the same time, for other samples, a reactivation could be observed. An increase of enzyme activity could be detected for samples C1 and S1 in the 2nd month, and for S2 in the 6th month. However, these changes could be proved mathematically, but for the low enzyme activity values these were not significant for the quality changes of the product.

Based on these observations it should be applied on a milder blanching treatment in frozen green pea processing technology when a shorter storage has to be considered (3−6 months), and the raw material requires a more energetic blanching being processed for a storage period of 12 month.

2.2. Effects of blanching time and temperature on the peroxidase enzyme formation

The blanching method selected for the bucket blanching equipment was water blanching. The changes during storage time were controlled by measurements of peroxidase enzyme activity, 8-times during the one-year storage period. The test results are presented in Table 3. Analysis of variance has been given in Table 4.

Table 3

Effect of blanching temperature and time on the peroxidase activity in green peas

Blanching parameters		Duration (months)							
temperature (°C)	time (min)	0		1		2		3.5	
		Residual POD activity (U g^{-1})							
		$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
82	3'50"	6395	618	4856	264	2538	137	4533	85
86	3'50"	331	11	317	60	325	33	219	17
90	3'50"	465	92	366	47	291	25	370	24
90	3'10"	362	60	187	52	195	94	337	20
90	2'15"	647	91	527	91	658	98	744	30

Blanching parameters		Duration (months)							
temperature (°C)	time (min)	5		7		9.5		12	
		Residual POD activity (U g^{-1})							
		$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
82	3'50"	3703	100	5030	632	2871	25	2594	258
86	3'50"	341	36	289	10	194	65	331	38
90	3'50"	460	15	382	10	127	20	137	41
90	3'10"	386	48	280	8	253	26	167	34
90	2'15"	715	12	530	27	621	7	612	7

Blanching was carried out in a water blanching equipment
Storage temperature: −20 °C
Number of parallel measurements: n = 3

Table 4

Two-way variance analysis for the investigation of the effects of blanching time and temperature

Blanching parameters		Duration of frozen storage (months)								Independently from storage time
temperature (°C)	time (min)	0	1	2	3.5	5	7	9.5	12	
82	3'50"	a	a	a	a	a	a	a	a	a
86	3'50"	c	c	c	d	d	d	cd	c	c
90	3'50"	bc	c	cd	c	c	c	d	d	c
90	3'10"	c	d	d	c	d	d	c	d	c
90	2'15"	b	b	b	b	b	b	b	b	b

Effect of treatment related to each other at 99% probability level in function of the blanching method
Formation of groups of treatment level in function of storage time

Storage time (months)	Blanching temperature and time 82 °C 3'50"	86 °C 3'50"	90 °C 3'50"	90 °C 3'10"	90 °C 2'15"
0	a	a	ab	ab	abc
1	b	a	cd	def	d
2	d	a	d	f	bcd
3.5	b	bc	bcd	abc	a
5	c	a	a	a	ab
7	b	ab	abc	bcd	cd
9.5	d	c	e	cde	bcd
12	d	a	e	ef	bcd

Non-significant differences were observed in the enzyme activity compared to the control sample. The statistical evaluation did not show any differences, with the exception of the blanching temperature of 82 °C. From the point of view of quality preservation, the enzyme activity for green peas blanched with these parameters was high, the blanching value insufficient and the product poorly storable. Other treatments produced an adequate blanching effect, based on the residual POD activity.

The effect of blanching temperature (82 °C, 86 °C, 90 °C) on peroxidase enzyme activity was observed to be different on a probability level of 99% only in case of the temperature of 82 °C. This enzyme activity was a multiple of the other two ones. As the inactivating effect of blanching temperatures of 86 °C and 90 °C resulted in identical POD activities, it can be suggested, that the heat sensitive peroxidase enzyme fraction was probably inactivated in the temperature range of 82–86 °C.

During the analysis of the influence of blanching time on peroxidase activity, a significant difference was observed for the blanching time of 2'15", but this treatment could produce sufficient residual enzyme activity, as well. Shorter treatments at higher temperatures caused slighter inactivation than longer ones at low temperatures.

During storage, the peroxidase enzyme activity changed its behaviour compared to that described in the study of the effects of the blanching methods on enzyme activity. The raw material qualities used for the treatments were different in the two assay series. For the second occasion, peas were more tender, thus a milder treatment was enabled by the parameters selected. The values measured directly after blanching and freezing were higher than those of the first assay. The initial enzyme activity was similar to that of sample W85 in the 0. month of storage.

The enzyme activity decreases slightly during storage, but the presence of heat stable isoenzymes, enzyme regeneration and enzyme activation can be observed. The homogeneity test performed for the variance analysis does not enable to draw any unambigous conclusion. Though there were found some significant differences, any

relation to the parameters of blanching could not be stated. With the exception of the enzyme activity of green peas blanched at the temperature of 82 °C, in the 5. month of storage the POD activity approached the initial value in all other treatments. After a more intensive heat treatment, the increase of enzyme activity was more rare during the storage period. In case of a lower residual enzyme value than 5–6% of the initial value, there was a minimal reactivation observed during storage.

2.3. Relation between pea diameter and peroxidase enzyme activity

On base of the data in Table 1, when the blanched green peas were separated into two groups according to their size, it has been found that in greater pea sizes a higher POD activity decrease has been caused by identical treatment, compared to smaller sizes. This difference is not too big and can be observed only for certain blanching treatment (S2, C2, C1). It could be imagined, that for these not so well-developed green peas, the binding of the enzyme in parts of skin and fibre was stronger.

In the industrial processing of deep-frozen green peas, the raw materials are sorted into groups of four sizes. It has been tested how in the four size categories enzyme activity changed after blanching, for pea diameters showing small variations. The test results have been summarized in Table 5.

In consideration of our knowledge on heat penetration, a decrease of POD activity could have been awaited with the increase of pea diameter. On base of the results of one way variance analysis, there was a difference proved statistically between enzyme activity values of peas with 6–8, 8–9 and above 10 mm seed diameters. The enzyme activity values of the size fraction 9–10 mm do not show any difference. Two groups could be separated with complete certainty, that below and above 10 mm pea diameter. In the interest of an adequate quality, the uniform value of peroxidase enzyme activity can be obtained after a sorting according to pea size with the application of various blanching parameters. This technological change requires further certification with new investigations.

Table 5

Relationship between residual POD activity in blanched green peas and pea diameter

Pea diameter (mm)	POD activity (U g^{-1}) $\bar{x}$	±s	VA[a]
6–8	127	41	a
8–9	191	6	b
9–10	83	5	a
above 10	328	25	c

Blanching parameters in water: temperature 95 °C, time 4 min

[a]Groups differing significantly at 95% probability level

Table 6

Correlation matrix between POD enzyme activity and ripening characteristics

Name	F	SZ	E	N	POD
Finometer degree (F°)	–	0.6760 0.0000	0.6842 0.0000	−0.4652 0.0084	−0.0010 0.9956
Pea diameter (SZ) (mm)		–	0.9592 0.0000	−0.0960 0.6073	−0.3130 0.0865
Mass of 1000 peas (E) (g)			–	−0.0694 0.7107	−0.3595 0.0470
Moisture content (N) (g per 100 g)				–	−0.4001 0.0257
Peroxidase activity (POD) ($U\ g^{-1}$)					–

Number of sample elements: 31
upper figure: correlation coefficient
lower figure: probability of the correlation coefficient being zero

2.4. Relationship between maturity and peroxidase enzyme activity of green peas

The two kinds of experiments described in para. 2.3. do not make possible a general conclusion. Further experiments were needed to establish whether maturity or heat penetration had a greater effect on enzyme activity. Beside the pea diameter, which characterizes ripeness, other ripening characteristics as applied in growing technology were compared to changes in enzyme activity. Mathematical-statistical methods were employed to find overall relationships and such applying to individual varieties between properties characterizing ripeness and POD activity. Appropriate functions were sought for. However, the properties investigated did not fit either linear or exponential or reciprocal functions. The correlation matrix between two variables each is shown in Table 6.

The last column of the Table shows clearly that no close relationship exists between POD activity and any of the ripeness indicators, the correlation coefficients were, in all cases, below 0.5. No close correlations were found either between individual pairs of ripeness indicators. The value of r was 0.95 only for the relationship between the mass of 1000 peas and pea diameter. Peroxidase activity is no ripeness indicator for green peas. Peroxidase activities of green peas of identical tenderness but belonging to different varieties were tested as well. Quality acceptance of green peas is carried out on the basis of tenderness, and peas of the same tenderness are heat treated under identical blanching conditions. The results of

the homogeneity test obtained by analysis of variance for the enzyme activities of the individual varieties treated around 100 °T are shown in Table 7.

Table 7

Variation of peroxidase enzyme activity with the variety

Varieties tested	Number of sample elements	Mean activity ($U\ g^{-1}$)	Identity levels
Maxigolt	6	30 866.167	*
Ujmajori	6	39 723.333	**
Margit	5	39 929.600	**
Booker seed	6	48 645.167	**
Polar	3	66 273.333	**
Ave	5	79 004	*

The enzyme activity values of the six varieties tested show significant differences. The varieties Maxigolt, Ujmajori and Margit differ significantly from the varieties Polar and Ave. Booker seed shows to be a borderline between the varieties Margit and Polar. In case of mixing the varieties of green pea raw material, great differences in enzyme activity can provide heterogeneous quality of the product. It is quite reasonable to consider this experience during processing and to choose the blanching conditions in accordance to variety combinations in the knowledge of enzyme activity.

3. Conclusions

Striving for total enzyme inactivation in blanching is not advisable. The technology applied in the industry indicates overblanching. On the basis of the experimental results we find it possible to alter the parameters of heat treatment. Inactivation of the heat sensitive fraction of peroxidase takes place around 82–85 °C. If residual enzyme activity does not exceed 5–6% of the initial value, reactivation during storage is minimal, maintenance of green pea quality will be satisfactory.

There is no unambigous relationship between peroxidase enzyme activity and degree of ripeness, however, peroxidase enzyme activity shows considerable variations with the variety. When selecting the method of blanching, its temperature and duration, it is essential to know the level of the peroxidase enzyme activity. Reducing the blanching parameters is only possible in those plants where the technical level of the equipment applied permits quick regulation. The control of blanching would require the introduction of a quick and reliable method of control in the industry as well.

Our experimental work was aimed at improving the quality of green peas destined for quick freezing, it can, however, contribute also to improve economic indices such as saving in energy and water consuption.

*

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A CORRELATION STUDY BETWEEN PROTEIN CONTENT AND SOME PHYSICAL TRAITS OF RICE GRAIN WITH THE AMINO ACIDS COMPOSITION

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This investigation was carried out to study the relationships between protein content and some physical traits of rice grain i.e. (grain length, grain width, grain shape, length/width ratio and 100 grain weight) with the essential and non-essential amino acids composition. Seven Egyptian and exotic rice varieties namely, Giza 171, Giza 172, Giza 175, Giza 176, Giza 181, IR 28 and IR 19743-46 were grown in 1989 and 1990 seasons and used to determine the studied traits.

The results revealed that protein content was positively correlated with grain length and negatively correlated with grain width and 100 grain weight. Protein content was neither phenotypically correlated with essential amino acids nor with non-essential amino acids.

Furthermore grain length and grain shape were significantly and negatively correlated with lysine, arginine and histidine and positively correlated with tryptophan. However, negative correlation coefficients were estimated between grain width with tryptophan and between 100 grain weight with phenylalanine + tyrosine, only.

Keywords: rice, protein content, amino acids, physical traits

The nutritional status of a variety is mainly dependent on its protein content. Rice protein is nutritionally superior to most of the other food cereals in terms of amino acids composition. The major limitations are that it is available in low quantities and the most part of it is lost in milling. Thus, despite its lower protein content (7%) milled rice has as high utilizable protein as the other cereal staples. Besides, most rice diets are not limiting in lysine. In addition, milled rice is essentially free of antinutrition factors (JULIANO, 1990). Accordingly, protein content indexes the nutritional value of milled rice and the nutrition value improved by improving the essential amino acids pattern of the protein content.

One of the interesting problems for practical breeding of rice is to explore the possibility of combining these traits which contribute to the higher grain production with that of best grain quality and high nutritional value. The difficulty that faces the breeders during the selection procedures is how to select a line with high protein content. The only way is to ascertain a correlation between some of the physical traits

of rice plant or rice grain with protein content and amino acids composition which need complicated methods for determination.

In studying the correlation between protein content and grain weight, TANAKA (1971) found significant positive correlation for protein content with single grain weight. However, MOHANTY and REDDY (1972), HILLERISLAMBERS and co-workers (1973), VILAWAN and SIDDIQ (1973) and HSIEH and KUO (1982) reported that the correlation between protein content with 100 grain weight was negative. On the other hand, VIJAYACHANDRA and MOHANTY (1977) indicated that this type of correlation was not significant.

Because of the variation in protein content by as much as six percentage points within a variety, only a few amino acids are affected by protein changes. For low-protein and high-protein, milled rice of eight varieties, the mean lysine contents were respectively 4.35 and 3.66 ($r = -0.66^{**}$) and mean tyrosine contents were 2.35 and 3.34 ($r = +0.69^{**}$) and mean glutamic acid contents were 21.0 and 23.2 ($r = +0.57$) (CAGAMPANG et al., 1966). For 16 samples of milled rices with 5.6–10.8% protein, protein content correlated negatively with lysine, threonine and methionine and positively with tyrosine, arginine and leucine (JULIANO et al., 1964). For 18 rough-rice samples of RD 7 variety ranging in protein content from 4.1–11.5% it was found to be correlated negatively with lysine ($r = -0.89^{**}$), cystine ($r = -0.92^{**}$), methionine ($r = -0.91^{**}$), threonine ($r = -0.87^{**}$), glycine ($r = -0.94^{**}$) and alanine ($r = -0.84^{**}$) and correlated positively with tyrosine ($r = 0.64^{**}$), phenylalanine ($r = 0.58^{*}$), leucine ($r = 0.58^{*}$) and glutamic acid ($r = 0.78^{**}$). The values of most coefficients of Thai varieties showed correlation between amino acid content and protein content. (EPPENDORFER et al., 1983). VILLAREAL and JULIANO (1978) reported that, the lysine and the protein content were not significantly correlated ($r = 0.16$). A detailed study of brown and milled rice samples verified that the negative correlation between the protein content and the lysine content of protein exists only below 10% protein for different varieties (I.R.R.I., 1973).

Less is known about the relationship between protein content and the physical traits of rice grain with the amino acids compositions such as grain length, width and shape as well as 100 grain weight. The present investigation was carried out to study the correlation coefficients between protein content and some physical traits of rice grain with the amino acids composition.

1. Materials and methods

The present investigation was carried out at the farm of Rice Research and Training Center (R.R.T.C.) Sakha, Kafr El-Sheikh, Egypt during 1989 and 1990 rice growing seasons. The chemical analysis for protein content and the amino acids

composition of the tested varieties were done at Biochemistry and Food Technology Department, Technical University of Budapest, Hungary.

Seven Egyptian rice varieties namely, Giza 171, Giza 172, Giza 176 (Japonica), Giza 181, IR 28, IR 19743-46 (Indica) and Giza 175 (Indica/Japonica) were used for this study and were grown in a randomized block design experiment with three replicates in the two seasons of study. Plants of each variety were individually transplanted at 20 cm × 20 cm spacings. A basal dose of 40 kg N and 30 kg P_2O_5 was given. Samples of grains were taken randomly and tested for the physical traits (grain length, grain width, grain shape (L/W ratio) and 100 grain weight according to KHUSH and co-workers (1979). The protein content of rice grains was done by estimating the total nitrogen by micro-Kjeldahl method as described in A.O.A.C. (1965) and multiplying the total nitrogen by 5.95 factor. Amino acids composition were analysed by the acid hydrolysate of ground samples using automatic amino acid analyser type AAA 881 Mikrotechna as described by SPACKMAN and co-workers (1958). Tryptophan was photometrically determined in alkaline hydrolysates according to FRIEDMAN and FINLEY (1971).

Phenotypic correlation coefficients for physical traits of grains with protein content and amino acids compositions were based on the method of SNEDECOR and COCHRAN (1968).

2. Results and discussion

2.1. Means of the physical traits of the grain of some Egyptian rice varieties

The present investigation determined the physical traits of rice grain i.e. grain length, grain width, grain shape and 100 grain weight in the two seasons of study. It is clear from Table 1 that there were a slight differences between the means of these traits in the two seasons of study.

The grain length ranged between 7.2 mm to 7.5 mm in 1989 and from 6.98 mm to 7.1 mm in 1990 for the Japonica group. The Indica varieties varied from 9.3 mm to 9.5 mm and from 8.81 mm to 9.01 in the two seasons of study. The same trend was observed for grain width character. These results affected also the values obtained for grain shape character (length/width ratio). On the other hand 100 grain weight differed from 1.85 g (for Giza 175) to 2.46 g (for IR 28) and from 2.1 g (for Giza 175) to 2.64 g (for Giza 176) in the two seasons, respectively. These results indicated that these traits might be affected by environmental factors.

Table 1

Means of some physical traits of the grain for the Egyptian rice varieties in 1989 and 1990 season

Group	Variety	Grain length (mm)		Grain width (mm)		Grain shape		100 grain weight (g)	
		1989	1990	1989	1990	1989	1990	1989	1990
Japonica	Giza 171	7.50	7.11	3.00	2.76	2.50	2.50	2.34	2.15
	Giza 172	7.20	6.98	3.30	3.04	2.18	2.20	2.39	2.47
	Giza 176	7.40	7.05	3.30	2.88	2.24	2.50	2.31	2.64
Indica	Giza 181	9.30	8.96	2.60	2.34	3.58	3.75	2.41	2.60
	IR 28	9.50	9.01	2.60	2.34	3.65	3.75	2.46	2.55
	IR 19743	9.50	8.81	2.70	2.66	3.52	3.31	2.41	2.13
Indica/ /Japonica	Giza 175	7.40	7.26	3.30	2.69	2.24	2.50	1.85	2.10

2.2. Means of the essential, non-essential amino acids composition and protein content of some Egyptian rice varieties

Table 2 presents the means of the essential and non-essential amino acids composition of the Egyptian rice varities in 1989 and 1990 seasons. It is clear that the values of the essential amino acids increased in 1990 in comparison to 1989 for all the varieties. Moreover, the rice variety Giza 171 (Japonica) had the highest values of these amino acids, while the lowest values were determined in IR 28 and IR 19743-46 (Indica) varieties. Regarding the non-essential amino acids, the results showed that the mean values differed in the two seasons of study. The highest values were determined in Giza 171 variety in both seasons for most of these components. These results were in agreement with EL-KADY and co-workers (1991).

Protein content was determined in the varieties and shown in Table 2. The data showed that protein content increased in the second season 1990 in most of the varieties. In 1989 it ranged between 6.28% and 8.70% for Giza 171 and IR 19743-46, respectively, while in 1990 it varied from 7.0% (for Giza 172) to 10.1% (for IR 19743-46). In general the highest values were determined for the Indica group and the Indica/Japonica variety (Giza 175). Same results were reported by EL-KADY and co-workers (1991).

2.3. Correlation coefficients between protein content and some physical traits of rice grain with the essential and non-essential amino acids composition

It is shown in Table 3 that protein content was positively correlated with grain length and negatively correlated with grain width and 100 grain weight. The observed negative correlation of protein content and 100 grain weight were in harmony with the information reported earlier concerning the disproportionate quantity of protein located in the outer layers and in the embryo of rice grain. These results were in agreement with those reported by MOHANTY and REDDY (1972), HILLERISLAMBERS and co-workers (1973), VILAWAN and SIDDIQ (1973) and HSIEH and KUO (1982). On the other hand, no significant coefficients were estimated between protein content and the essential amino acids composition. Despite there were high magnitudes of such estimate with some of the essential amino acids (0.463-0.457 and −0.342 for tryptophan, lysine and leucine, respectively), these values were not significant, same results were obtained by VILLAREAL and JULIANO (1978). While, significant correlation coefficients between protein content and some amino acids composition were found by CAGAMPANG and co-workers (1966) and EPPENDORFER and co-workers (1983).

Table 2

Protein and amino acids composition of some Egyptian rice varieties in 1989 and 1990 seasons
(g per 16.8 g N)

Amino acids	Group: Japonica						Indica						Indica/Japonica	
	Giza 171		Giza 172		Giza 176		Giza 181		IR 28		IR 19743		Giza 175	
	1989	1990	1989	1990	1989	1990	1989	1990	1989	1990	1989	1990	1989	1990
Essential amino acids														
Leucine	9.54	11.73	8.24	9.43	6.86	7.73	7.84	7.76	8.90	7.35	4.54	9.42	8.48	8.88
Isoleucine	4.25	9.33	3.69	4.00	3.10	2.88	3.30	4.46	3.54	3.37	3.04	3.49	4.22	3.48
Lysine	5.70	7.60	3.59	7.57	2.62	4.24	3.38	4.32	2.24	4.10	2.69	4.30	3.55	4.83
Methionine + cystine	2.41	4.93	2.52	3.86	2.76	3.79	2.69	4.73	4.25	4.10	2.70	3.03	2.90	3.93
Phenylalanine + tyrosine	10.80	13.74	8.08	9.00	8.31	9.24	9.22	9.05	10.58	8.44	9.80	10.12	9.28	11.68
Threonine	5.08	5.47	3.88	4.00	4.05	3.03	4.05	3.92	3.90	3.37	3.45	4.19	4.33	3.82
Valine	6.33	8.53	5.38	4.71	4.56	4.70	5.10	5.68	5.06	4.82	4.66	5.70	5.77	5.73
Tryptophan	1.28	1.66	1.24	1.86	1.29	1.55	1.95	1.70	1.80	1.41	1.76	1.33	1.64	1.25
Non-essential amino acids														
Alanine	6.58	8.40	4.86	4.86	4.99	5.15	5.48	5.54	5.86	5.78	4.80	5.58	5.18	6.18
Arginine	10.12	12.20	7.63	10.00	7.22	8.79	7.52	7.84	7.80	7.95	7.31	8.37	6.67	10.11
Aspartic acid	14.04	16.53	10.22	11.71	12.32	9.39	13.08	10.14	15.06	10.12	11.08	9.42	10.47	11.57
Glutamic acid	22.83	31.60	21.85	18.57	17.68	20.91	19.81	22.43	22.24	18.07	18.07	21.40	23.27	22.70
Glycine	4.96	8.13	4.29	3.86	4.55	4.85	4.59	4.05	4.85	3.74	3.81	4.42	4.89	5.51
Histidine	3.99	4.53	3.86	4.14	2.63	2.27	2.67	2.30	2.81	2.53	2.65	2.21	2.96	2.70
Proline	5.07	7.60	4.03	5.29	3.96	4.70	4.38	4.46	4.82	4.94	4.43	4.65	4.55	4.94
Serine	7.67	8.53	3.85	6.29	4.72	5.00	4.56	6.07	5.43	5.42	4.73	5.23	3.38	6.29
Protein content (%)	6.28	7.50	7.18	7.00	6.30	6.60	7.70	7.40	8.49	8.31	8.70	10.10	8.37	8.90

Table 3

Linear correlation coefficients among physical traits, protein content with essential and non-essential amino acids composition of some Egyptian rice varieties

Characters	Protein content	Essential amino acids composition: Leucine	Isoleucine	Lysine	Methionine + cystine	Phenylalanine +tyrosine	Threonine	Valine	Trytophan
Protein content	1.000	-0.342	-0.308	-0.457	-0.239	0.134	-0.235	-0.099	0.463
Grain length	0.585*	0.026	-0.285	-0.622*	0.345	-0.142	-0.352	-0.269	0.874**
Grain width	-0.562*	-0.143	0.086	0.496	-0.357	-0.092	0.138	0.051	-0.819**
Grain shape	0.475	0.034	-0.222	-0.587*	0.389	-0.093	-0.296	-0.216	0.856**
100 g weight	-0.612	-0.382	-0.285	-0.268	0.287	-0.607*	-0.432	-0.517	0.318

	Protein content	Non-essential amino acids composition: Alanine	Arginine	Aspartic acid	Glutamic acid	Glycine	Histidine	Proline	Serine
Protein content	1.000	-0.160	-0.499	-0.329	-0.166	-0.277	-0.439	-0.200	-0.358
Grain length	0.585*	-0.072	-0.599*	-0.069	-0.312	-0.456	-0.577*	-0.261	-0.069
Grain width	0.562*	-0.138	0.513	-0.104	0.101	0.248	0.480	0.062	-0.118
Grain shape	0.475	-0.002	-0.572*	0.005	-0.255	-0.389	-0.548	-0.202	0.006
100 g weight	-0.612	-0.275	-0.098	0.039	-0.505	-0.460	-0.159	-0.295	-0.036

*Significant and **highly significant at P = 0.05 and P = 0.01 probability level, respectively

Furthermore Table 3 revealed that protein content was insignificantly correlated with the non-essential amino acids composition. High values were estimated between protein content with arginine (−0.449), histidine (−0.439), serine (−0.358) and aspartic acid (−0.329), however, these coefficients were not significant. These results were in harmony with CAGAMPANG and co-workers (1966), while EPPENDORFER and co-workers (1983) found that protein content was correlated negatively with alanine and glycine and positively with glutamic acid.

2.4. Correlation coefficients between the physical grain characters with the essential and non-essential amino acids composition

Evidently Table 3 indicated that grain length and grain shape (length/width ratio) were negatively correlated with lysine, moreover highly significant correlation coefficients among these two traits with tryptophan. These correlation coefficients with tryptophan were as high as 0.874 and 0.856 for grain length and grain shape respectively. On the contrary, grain width character was highly significantly and negatively correlated with tryptophan, only.

Table 3 further revealed that insignificant correlation coefficients were obtained between 100 grain weight and the essential amino acids composition except with phenylalanine + tyrosine. These means that the studied physical traits might be independently inherited from most of the essential amino acids composition.

Data in Table 3 emphasized that negative and significant phenotypic correlation coefficients (−0.599 and −0.577) were estimated between grain length with arginine and histidine, and between grain shape with arginine (−0.572). Table 3 further revealed that no significant phenotypic correlation coefficients were obtained between the physical traits of rice grain with most of the non-essential amino acids composition.

Finally, the present investigation revealed that there were significant correlation coefficients between protein content and the physical traits of the rice grain with some essential and non-essential amino acids composition. These observed phenotypic correlation may be due to linkage or to pleiotropic gene action. However, the estimated insignificant values of the correlation coefficients between the same traits with most of the amino acids concluded the possibility of combining the best physical traits of the rice grain with the desirable amounts of such amino acids to improve the nutritional value of the commercial rice varieties.

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EFFECT OF SHORTENED FERMENTATION TIME ON ACID DEVELOPMENT AND SUGAR METABOLISM OF COCOA BEANS

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Beans were fermented in sweat boxes for 5 days without turning over and dried in a mechanical drying machine. The final pH of the bean's mass was 4.3. Acetic acid in the pulp reached a maximum level on the third day (13.4 mg per 10 g beans) and decreased the day after, whilst lactic acid level was maximal (10.2 mg per 10 g beans) on the fourth day of fermentation. Likewise, acetic acid in the cotyledon reached a maximum level on the third day of fermentation. The concentration of lactic acid was higher than any other organic acids during drying. It was found that SMC-1B beans may give a better flavour by virtue of its high pH. Sugar was metabolized rapidly from the early stages of fermentation and lactic and acetic acids were the major products.

Keywords: anaerobic fermentation, acidity, sugars, cocoa beans

Cocoa is the third major crop in Malaysia after rubber and oil palm. Production of good quality cocoa is enhanced by proper techniques of fermentation as the process results in the formation of precursors which are essential for good flavour and aroma. It also brings about a suppression of astringency and bitterness (DIMICK, 1983; BIEHL, 1984).

Changes in sugar, ethanol, acetic acid and lactic acid levels have been reported by a number of researchers (FORSYTH & QUESNEL, 1963; LOPEZ & QUESNEL, 1973; GRIFFITH, 1961) and are best exemplified by the work of CARR and co-workers (1979). The data of CARR and co-workers (1979) although indicate trends in cocoa fermentation, does not characterise the changes required for the optimum fermentation of cocoa beans since acid tainted chocolate was produced from all beans fermented.

The degree of aeration of fermenting pulp is a key factor in controlling microbial activity. The effect of aeration is influenced by whether or not the beans are turned over (QUESNEL, 1968). Increased oxygenation resulted in higher maximum temperatures during fermentation due to the exothermic nature of microbial oxidation of ethanol and acetate. Other researchers (DOUGAN et al., 1981; HENDERSON, 1980) have investigated the role of aeration in heap or box fermentation of cocoa beans. These studies indicate that aeration was necessary to enhance acetic acid production by microorganisms.

Cutting short of the normal 6 days of fermentation time would of course save labour cost and time. However, the question that arises was whether the beans were sufficiently fermented. The acid or pH may be an important factor that could determine the bean's quality. The presence of excessive residual acidity may also affect the development of chocholate flavour. A high degree of acidity is usually associated with pH 5.0 or less in dried beans (MUSA & SAID, 1988). The objective of this study is to relate the acidity condition during 5 days trial anaerobic fermentation and also to elucidate acid level on beans that have been graded as SMC-1B or SMC-1C. The acid and total sugar concentrations were determined and their importance discussed.

1. Materials and methods

Fermented cocoa beans of mixed Sabah hybrid harvested from ripe fruits were obtained from an estate in Peninsular Malaysia. Samples were preserved intact in plastic bags and kept cool in ice during transportation to the laboratory to minimise microbial activity. At the estate, the bean's mass were fermented on a trial basis in a fermentation box (150 cm × 170 cm × 39 cm) for 5 days without turning over. Approximately 30 g of bean's sample were taken from the middle of bean's mass which was 12 cm from the top at every 24 h interval. The beans were dried in a mechanical rotary operated drying machine for about 7 h at 65 °C until the moisture of the beans was approximately 8%. At the final grading of the beans they were given code names such as SMC-1B and SMC-1C by the estate after the fermentation process.

Bean samples of approximately 10 g of both cotyledon and pulp as well as including those that have been graded were crushed separately using a MX-20 GN National Blender, (Matsushita Electric Industrial Co., Ltd., Osaka, Japan) for 2–3 min in 100 cm^3 distilled water containing 0.2% (w/v) benzoic acid. The pH of the suspension was measured in duplicates with a pH meter (Model 630, Fisher Scientific Co., USA). The suspension was then centrifuged at 18 000 g for 30 min at 4 °C (IEC-B-20A Centrifuge, International Equipment Co., USA) and the supernatant fluid obtained was first filtered through a sep-pak C_{18} cartridge and then through a millipore filter (0.45 μm pore size membrane filter). Samples were analysed for total sugars and acid level. Sugars were determined by total carbohydrate method (DUBOIS et al., 1956). Ten μl of sample solutions were injected into an HPLC column (HPLC organic analysis column: Aminex ion exclusion HPX-87H, 1084B Liquid Chromatograph, Hewlett-Packard, USA) with a variable wavelength detector at 210 nm. Oven temperature of the HPLC was programmed at 85 °C and the solvent was maintained at a flow rate of 0.9 cm^3 min^{-1}. The solvent used was a mixture of 0.05 mol l^{-1} orthophosphoric acid and distilled water (2.8 : 100, v/v). The pH of the solvent was adjusted to 2.7. Volatile and non-volatile acids were determined from peak areas by reference to a standard curve prepared with known amounts of pure acids. All samples were measured in triplicates.

2. Results and discussion

2.1. pH changes

The overall pH values obtained from this fermentation trial were very low. At 0 day the pH of the cotyledon observed was 6.40 and decreased to 4.35 on the fifth day, whereas the pH of the pulp increased from 3.75 to 4.55 on the final day (Fig. 1). The pH of the pulp increased after the 3rd day probably because less sugar was being metabolised. A lower sugar content will decrease the ethanol production and as a result pH values become higher. The acids produced in the pup diffused slowly into the cotyledon thus becoming more acidic. However, when the beans were dried most of the volatile fatty acids especially acetic acid would disappear. This would further increase the pH value.

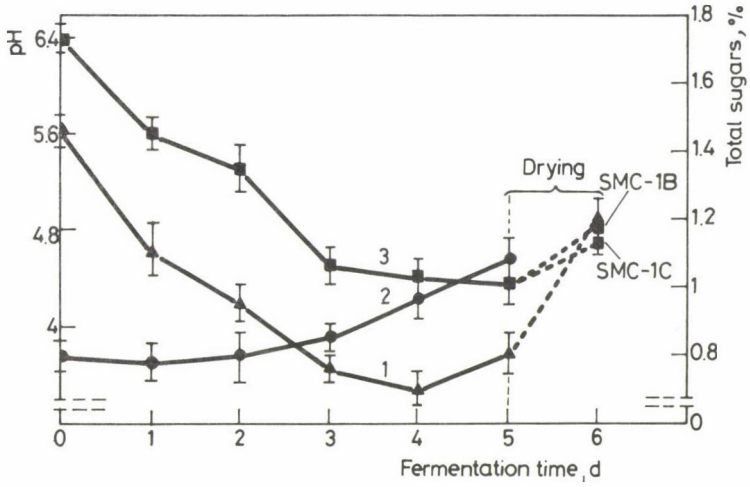

Fig. 1. pH and total sugars profile of cocoa beans during anaerobic fermentation. 0: unfermented beans, 1–5: fermented beans, 5–6: drying. Samples were taken at 12.30 h each day. Each sample was assayed in duplicates. 1: Total sugars, 2: pulp, 3: cotyledon

2.2. Total sugars

At the start of fermentation, there was a high concentration of total sugars present and this was broken down and hydrolyzed to simple sugars, probably glucose and fructose. Because of low pH, high sugar content and low oxygen supply in the pulp during the initial stages of fermentation, yeast activity was favoured. As shown in Fig. 1, sugar was rapidly reduced after 24 h fermentation time and continued to

decrease until the fourth day. It is most likely that the drastic drop in the sugar content was due to the assimilation of sugar by yeast. Since the bean mass was not turned over, the top surface of the mass would cause the yeast to proliferate and metabolize the sugar to carbon dioxide and water. Less aeration at the bottom would probably encourage the lactic acid bacteria to grow and convert sugar into lactic acid.

The increase in the total sugar content is due to the decrease in the moisture content during drying. Analysis on a dry weight basis revealed that a significant amount of sugar was retained. This suggests that, depending on the physical treatments that the beans receive, pulp sugars can be retained and can contribute to the sugar fraction of cocoa beans.

2.3. Organic acids

As shown in Figs. 2 and 3, only four major organic acids were subsequently identified in the pulp and cotyledon. These acids were acetate, lactate, oxalate and citrate. Metabolism of citric acid and the formation of the lactic and acetic acids in the pulp caused the overall pH changes inside the bean. Citric acid is dissimilated to lactic acid and acetic acid as well as carbon dioxide by heterofermenters. In contrast, citric acid is broken down to acetone and carbon dioxide by homofermenters (WHITING & COGGINS, 1964). All these activities probably reduce the acidity during cocoa fermentation. The destruction of a strong acid like citrate will affect the pH more profoundly than the generation of a weak acid such as acetic.

According to CARR and co-workers (1979), acetic acid bacteria appear earlier in the superficial zones of fermenting mass and are more prevalent than at the centre. Therefore, acetic acid was distinguished by an early increase to a maximum level at 72 h and has a tendency to decrease thereafter in both pulp and cotyledon. Acetic acid developed in the pulp, possibly by acetic acid bacteria, progressively caused the pH to decline. The ANOVA showing the difference in the production of 4 acids in the pulp (Fig. 2) gives F value of 9.22 which is highly significant. On investigating the means over a 5 day period, acetic acid production is found to be greater than the rest. Likewise, the ANOVA which shows the difference in the production of similar acids in the cotyledon (Fig. 3) give a F value of 10.79 which is also highly significant. In this instance acetic acid appear to be greater than other organic acids.

Lactic acid in cocoa beans was produced by the lactic acid bacteria (CARR et al., 1980). Since the beans were not turned over, lactic acid continued to increase throughout the fermentation process. This was because lactic acid bacteria could produce lactic acid under both aerobic and anaerobic conditions. It was posssible that at the superficial layer, there was sufficient aeration from the early stages of fermentation to suppress almost completely the growth of lactic acid bacteria. As a

result lactic acid developed more on the fourth day. Lactic acid constituted the major product for both pulp and cotyledon after 5 days of fermentation (Figs. 2 and 3).

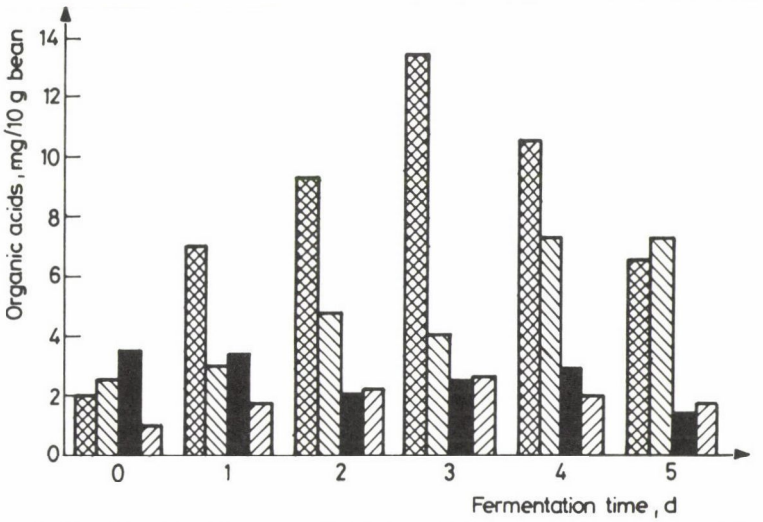

Fig. 2. Concentration of organic acids present in the pulp of cocoa beans during anaerobic fermentation. 0: unfermented beans, 1–5: fermented beans. Mean concentration was within 3% variation. ▨: Acetate, ▧: lactate, ■: citrate, ▨: oxalate

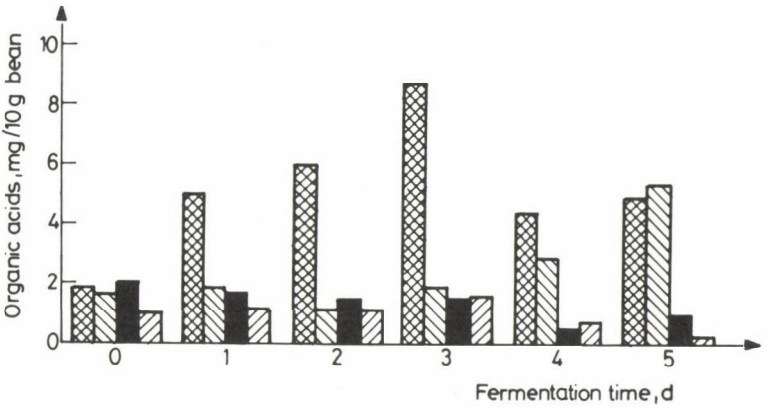

Fig. 3. Concentration of organic acids present in cotyledon of cocoa beans during anaerobic fermentation. 0: unfermernted beans, 1–5: fermented beans. Mean concentration was within 4% variation. ▨: Acetate, ▧: lactate, ■: citrate, ▨: oxalate

There were slight differences in the organic acid contents of SMC-1B and SMC-1C samples. The ANOVA shows the difference in the acid level between SMC-

1B and SMC-1C, and it gives an F value of 1.07 which is slightly significant. Quantitatively, acetic and citric acids were less prevalent in SMC-1B than in SMC-1C. The overall results indicate that the acidity in SMC-1C was slightly greater than in SMC-1B (Fig. 4). In this instance, cocoa grading done by the estate was right since grade SMC-1B would fetch a better price than SMC-1C.

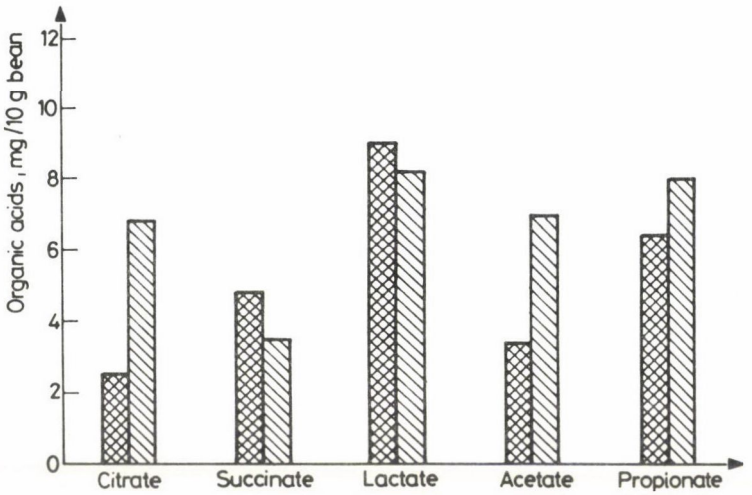

Fig. 4. Concentration of organic acids present in beans which have been graded as SMC-1B and SMC-1C for export. Mean concentration was within 1% variation. □: SMC-1B, □: SMC-1C

3. Conclusions

The effect of shortened fermentation time may bring about astringency and bitterness which is probably due to the presence of tannins or tannic acids because the beans were underfermented. Since the final pH of the bean mass was low (pH 4.3) it would grossly affect the development of chocolate flavour. For beans that have been fermented for 6 days with daily turning interval the pH achieved was 5.33 (ABDUL SAMAH et al., 1990). Although the flavour was found to be slightly weak it might have been worse for the underfermented beans. These flavours are due to the presence of an excessive amount of certain acids, especially acetic and lactic acids formed during fermentation. During manufacture acetic acid present in the dried beans will normally be reduced but not the non-volatile lactic acid. The presence of excessive residual acidity may also affect the development of chocolate flavour.

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PREPARATION AND FUNCTIONAL PROPERTIES OF RAPESEED PROTEIN PRODUCTS

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The mixture of three double zero rapeseed cultivars Lindore, Santana and Tandem grown in Hungary, were examined. The protein of raw rapesed meal showed two isoelectric points, the first at pH 3.2 and the second one at pH 7.2. The yield of protein isolate was 39.3% using two-step precipitation, while it reached to 68.3% when sodium hexametaphosphate was used as precipitation aid and single-step precipitation at pH 3.2. The heat treatments reduced the glucosinolates and phytic acid contents by about 47–93% and 9–42%, respectively.

Isolation of proteins reduced about 72–94% of glucosinolates and 73–91% of phytic acid contents for concentrate and isolate, respectively. Both protein isolate and concentrate have very good functional properties (NSI; water absorption; fat absorption and emulsifying capacity), excepting emulsifying activity and emulsion stability.

Keywords: rapeseed protein isolate and concentrate, glucosinolates, phytic acid, heat treatment, functional properties

In view of shortage in animal proteins in the world, oilseeds are becoming of increased importance as sources of edible proteins. Rapeseed ranks the fifth among the world's oilseed crops (soybean, cottonseed, peanut, sunflower and rapeseed). Some factors which favour the use of rapeseed protein in human nutrition are:

– The seeds contain about 25% protein while defatted meal has as high as 40% protein (on the dry weight basis);

– The meal protein has a very well balanced amino acid composition as compared to other plants, and is especially rich in sulfur-containing amino acids and lysine, the first limiting amino acid in legumes and cereals, respectively (GILLBERG & TÖRNELL, 1976 a,b).

Rapeseed protein having high nutritional value and satisfactory functional properties is well accepted.

There are some problems involved in the use of rapeseed in nutrition such as:

– The presence of glucosinolates in the meal, which on enzymatic hydrolysis (thioglyycosidase, also present in the seed) yield one or more aglucon products such as isothiocyanate; thiocyanate and nitrile (VAN ETTEN, 1980). Intact glucosinolates

and aglucon products cause thyroid enlargment and inhibition of growth in experimental animals;

- The presence of antinutritive substances such as phytic acid and polyphenols in the rapeseed meal is considerable.

In the present study, we suggest to eliminate undesirable components by two ways:

- Using heat treatment: a) Toasting at 110 °C for 1 h, b) autoclaving at 121 °C for 1 h and c) autoclaving at 121 °C for 1 1/2 h;

- Isolating the proteins a) preparation of protein isolate, b) preparation of protein concentrate. Also we study the effect of the latter treatment on the technofunctional properties of proteins.

1. Materials and methods

1.1. Meals

Three cultivars of double zero rapeseed are grown in Hungary; our sample was the mixture of these cultivars Lindore, Santana and Tandem. Defatted raw rapeseed meal and toasted rapeseed meal (110 °C for 1 h) were obtained from the Research Institute of Vegetable Oil and Detergent Industry, Budapest. Autoclaved rapeseed meals (121 °C for 1 h and 1 1/2 h, respectively) were prepared from raw rapeseed meal. All the rapeseed products were finely ground to pass a 400 μm screen.

1.2. Protein isolate preparation

1.2.1. Extraction of protein. The meal was dispersed in deionized water at room temperature and the pH of the dispersion was increased to 12.1 by addition of aqueous NaOH (40%) and shaken for 1 h. The final solid to liquid ratio was 1:20 (w/v). Insoluble materials were removed by centrifuging at 5000 r.p.m. for 15 min. The maximum portion of nitrogen extracted was 81.5%.

1.2.2. Precipitation of protein. The protein was precipitated from the clarified extract by adjusting the pH to definite values, and the precipitate was washed twice with distilled water and dried by adding acetone followed by keeping in oven under vacuum at 30 °C for 4 h. The nitrogen yield was calculated as 81.5% (total nitrogen in the dry protein isolate/total nitrogen in the extract).

1.2.3. Improvement of the yield. - To increase the yield we used sodium hexametaphosphate (8%) as a precipitation acid according to the method of GILLBERG AND TÖRNELL (1976a) followed by two-step precipitation of proteins.

- Sodium hexametaphosphate followed by single-step precipitation of proteins at pH 3.2.

1.2.4. Improvement of the colour. – In order to improve the colour the protein isolate was prepared by adding sodium metabisulfite to the extracting agent in the following proportion: 1% or 0.2%.

– Sodium metabisulfite 0.1% to the extracting agent followed by soaking the precipitate in 0.1% or 0.2% sodium metabisulfite for 1 h.

– Rapeseed protein isolate was prepared by adding 0.2% sodium metabisulfite to the extracting agent and washing the precipitate twice with ethanol 50% and once with distilled water according to the method of THOMPSON and co-workers (1982a).

1.2.5. "Low phytate" rapeseed protein isolate. A "Low phytate" rapeseed protein isolate was prepared by precipitation the protein at pH 4.7 according to the method of GILLBERG AND TÖRNELL, (1976b).

1.3. Protein concentrate preparation

The method of LIU and co-workers (1982) and modified by THOMPSON and co-workers (1982a) was used.

1.4. Analytical methods

Aqueous ether extract, ash, crude fiber, protein solubility profile were determined according to the method of A.O.A.C. (1980). Crude protein ($N \times 6.25$) content was determined by the Kjeldahl procedure using an automatic Kjel-Foss equipment (Model 16210, made in Denmark).

Non-protein nitrogen was determined by the method of BHATTY and FINLAYSON (1973), with the modification of NACZK and co-workers (1985).

Total carbohydrate was determined by Schoorl after hydrolysis for 3 h with HCL 2.5%.

Phytic acid was determined by the method of MAKOWER (1970).

Total glucosonolates content was determined by the method of MÖLLER and co-workers (1987) based on the colour reaction with palladium.

Different protein fractions were determined according to the method of OSBORNE (1924).

1.5. Functional properties

The pH values were determined using a 10% dispersion (w/v) of meal in distilled water.

Nitrogen solubility index (NSI) was determined at pH 7.0 according to A.A.C.C. (1969) methods as described by THOMPSON and co-workers (1982b). It was also determined in water and NaCl 1 mol l^{-1} at natural pH of products.

Water and fat absorption were measured by the methods of SOSULSKI (1962) and LIN and co-workers (1974), respectively.

Oil emulsifying capacity was determined by dispersing 0.5 g of the sample in 23 cm^3 of distilled water and homogenizing for 0.5 min at the highest speed (Ultra-Turrax homogenizer, Janke and Kunkel, TP 18/10 Staufen, i.Br., Federal Republic of Germany). Other steps were similar to those outline by BEUCHAT and co-workers (1975). Emulsifying capacity is expressed as cm^3 sunflower oil per gram sample. Emulsifying activity and emulsion stability were studied by the method of YASUMATSU and co-workers (1972) but emulsions were prepared at a final sample concentration of 3.5% (w/v).

2. Results and discussion

Composition and non-protein nitrogen content of rapeseed meals, protein concentrate and isolate are shown in Table 1. The results indicate that the rapeseed protein products contained high levels of protein and non-protein nitrogen. The percentages of non-protein nitrogen were 5.47–6.78 for meals; 1.15% for protein concentrate and 0.88% for protein isolate.

The effect of heat treatment on the protein solubility profile of rapeseed meals is presented in Fig. 1. Protein of raw rapeseed meal had two isoelectric points, similar to those reported for rapeseed meal (GILLBERG & TÖRNELL, 1976a,; ELNOCKRASHY et al., 1977 and NACZK et al., 1985). The first isoelectric point appears at pH 3.2 and the second at pH 7.2. The heated rapeseed meals have only one isoelectric point in the acidic region, in consequence of the heat treatment (NACZK et al., 1985).

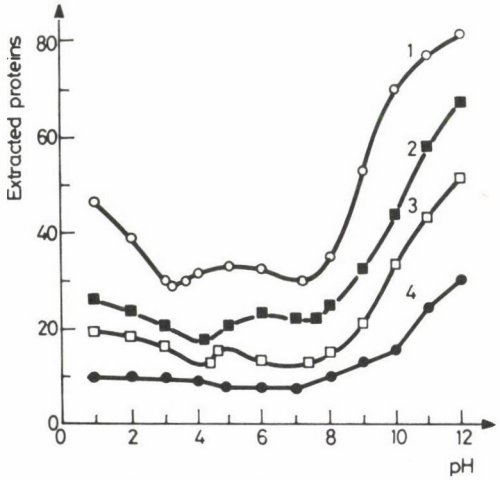

Fig. 1. Effect of heat treatment on the protein solubility profile of rapeseed meals. 1: Raw meal, 2: toasted meal, 3: autoclaved meal (1 h), 4: autoclaved meal (1.5 h)

Table 1

Chemical composition and non-protein nitrogen content of rapeseed products
(on dry weight basis)

Rapeseed products	Crude protein (N x 6.25) (%)		Oil (%)		Crude fiber (%)		Ash (%)		Carbohydrate[a] (%)	Non-protein nitrogen (% of crude protein)	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s		$\bar{x}$	±s
Raw meal	42.89	0.4	1.67	0.1	10.75	0.3	7.36	0.1	18.72	6.78	0.1
Toasted meal	43.17	0.4	2.07	0.2	11.12	0.4	7.21	0.1	17.89	6.04	0.1
Autoclaved meal, 1 h	42.12	0.3	2.29	0.1	11.49	0.4	7.69	0.1	18.95	5.47	0.1
Autoclaved meal, 1 1/2 h	42.08	0.3	1.77	0.1	11.55	0.4	6.75	0.5	17.12	5.97	0.1
Protein concentrate	89.42	0.1	0.17	0.02	0.10	0.03	7.83	0.1	1.11	1.15	0.1
Protein isolate	92.16	0.2	0.35	0.05	0.04	0.02	3.78	0.1	1.61	0.88	0.1

Mean values of three determinations
[a]Mean of two determinations

Table 2 shows the effect of pH precipitation and addition of sodium hexametaphosphate on the yield of protein. The protein exhibits an isoelectric point at pH 7.2 about 34.7% of total extracted nitrogen (81.5% of meal nitrogen) and another at pH 3.2 about 4.6% and the total yield about 39.3%. These results are in agreement with that of GILLBERG and TÖRNELL (1976b), they reported that the maximum yield in the second precipitation step was about 7% of the extracted nitrogen and the total nitrogen yield in two steps was slightly lower than 50%.

Table 2

Effect of sodium hexametaphospate, stepwise and single-step precipitations on the yield of rapeseed protein isolate

Sodium hexa-phosphate (%)	pH of precipitation stepwise and single step	Yield (%)	Total yield (%)
–	7.2	34.7	
			39.3
	3.2	4.6	
	7.2	15.2	
8			49.4
	3.2	34.2	
8	3.2	68.3	68.3
8	4.7	63.5	63.5

The low yields may be a consequence of the fact that rapeseed has a very complicated protein composition and contains proteins with widely different isoelectric points and molecular weights (LÖNNERDAL & JANSON, 1972). Furthermore, the high concentration of non-protein nitrogen remaining soluble when the proteins are precipitated at the isoelectric point reduced the yield of protein products (RUTKOWSKI, 1979). The addition of sodium hexametaphosphate led to increase the yield by about 26% using two-step precipitation, while the yield increased by about 74% when single-step precipitation was used at pH 3.2.

The effect of various treatments on the colour and yield of protein isolate is shown in Table 3. Addition of sodium metabisulfite to the extracting agent yielded a lighter coloured protein isolate than in its absence (THOMPSON et al., 1982a). The yield was drastically decreased at a high concentration of sodium metabisulfite (BLAICHER et al., 1983). The lightest colour was observed by a combination of the sodium metabisulfite and soaking treatment. The increase of sodium metabisulfite level in the soaking treatment yielded lighter coloured products with a slightly decreased yield of protein.

Table 3

Effect of sodium metabisulfite and other treatment on the colour and yield of rapeseed protein isolate

Sodium meta-bisulfite (%)	Treatment	Colour	Yield (%)
–	–	light black-brown	68.3
1.0	–	dark yellow	53.1
0.2	–	dark brown	66.9
0.2	ethanol 50% twice[a]	brown	65.7
0.1	soaking in 0.1%	light brown	64.3
0.1	soaking in 0.2%	light beige	63.6

[a]THOMPSON et al. (1982a)

Extraction of proteins from rapeseed products by the Osborne method is presented in Table 4. The major protein fractions in the rapeseed products are globuline and albumine, respectively. The heat treatment denaturated protein molecules and caused change in protein solubility. The extraction of proteins decreased gradually with the increase in the heating temperature. Both protein isolate and concentrate have a low albumine fraction because the pH of extraction of both proteins is near to their isoelectric points.

Protein solubility profile of rapeseed protein isolate and concentrate is presented in Fig. 2. The protein isolate and concentrate had a minimum solubility in the pH range between 4.4–4.7. The solubilities at pH 11 were 97 and 70% for isolate and concentrate, respectively. The solubility at pH 1 was 37.5 and 51% for isolate and concentrate, respectively. Both protein isolate and concentrate had only one isoelectric point because both proteins were precipitated by single-step precipitation.

Table 5 shows that raw rapeseed meal contains high and low levels of total glucosinolates and phytic acid, respectively, compared with those reported by LIU and co-workers (1982) and TZENG and co-workers (1988), while phytic acid content of raw rapeseed meal is similar to those noted by BLAICHER and co-workers (1983) and BINZHOU and co-workers (1990). Glucosinolates and phytic acid concentrations drastically decreased with increasing heat treatment. Among the treatments, heat treatments in an autoclave caused more reduction in phytic acid than treatment by toasting. Autoclaving might have a deesterification effect on phosphate residues in phytic acid.

On the other hand, isolation of the protein reduced glucosinolate content by almost 95%. SOSULSKI and DABROWSKI (1984) found that preparation of protein isolate by the alkaline method reduced 96% of the glucosinolates content of flour.

Table 4

Extraction of proteins from rapeseed products by Osborne-method

Rapeseed products	Protein fraction soluble %(w/w) in H_2O		NaCl (1 mol l^{-1})		Ethanol (75%)		KOH (0.2%)		Protein in residue (%)	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Rape meal	28.00	1.3	33.70	1.5	11.03	1.5	6.79	1.5	20.48	3.2
Toasted meal	15.56	1.4	19.03	1.5	8.62	1.5	6.92	1.5	49.85	2.9
Autoclaved meal, 1 h	6.87	0.5	12.50	0.3	5.11	0.3	3.35	0.3	72.17	1.1
Autoclaved meal, 1 1/2 h	0.87	0.3	5.07	0.3	2.98	0.3	2.28	0.3	88.77	0.3
Protein concentrate	5.68	0.1	44.69	0.3	9.55	0.1	5.51	0.1	33.39	0.1
Protein isolate	0.80	0.1	35.84	0.5	9.93	0.2	3.79	0.4	49.47	0.6

Mean of three determinations

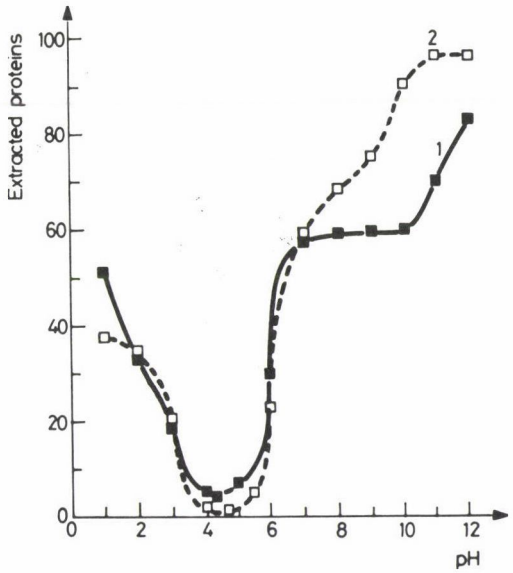

Fig. 2. Protein solubility profile of rapeseed protein isolate and concentrate. 1: Protein concentrate, 2: protein isolate

Table 5

Effect of heat treatment and isolation of protein on glucosinolates and phytic acid contents of rapeseed products
(On the dry weight basis)

Rapeseed products	Glucosinolates (μ mol g^{-1}) $\bar{x}$	$\pm s$	Reduction (%)	Phytic acid[a] (%)	Reduction (%)
Raw meal	35.41	0.8	–	3.27	–
Toasted meal	18.67	0.8	47.27	2.98	8.87
Autoclaved meal, 1 h	13.60	1.3	61.60	2.84	13.15
Autocalved meal, 1 1/2 h	2.28	0.4	93.56	1.88	42.51
Protein concentrate	9.83	1.3	72.24	0.86	73.70
Protein isolate	1.85	0.0	94.79	0.27	91.74

Mean of three determinations
[a]Mean values of two determinations

Isolated proteins had a low phytic acid content as compared to those reported by DEV and MUKHERJEE (1986) and TZENG and co-workers (1988). Furthermore the isolation reduced the phytic acid content in proteins almost by 92%, while BINZHOU

and co-workers (1990) reported that preparation of protein isolate by three-stage countercurrent extraction at alkaline pH reduced the one-third of the phytic acid content of defatted rapeseed meal.

Technofunctional properties of rapeseed products are presented in Table 6. NSI values of heated meals decreased gradually with increasing temperature, this effect is related to protein denaturation. Protein concentrate and isolate had a low NSI in water because of both proteins were near to their isoelectric points, while both proteins had a high NSI at neutral pH similar to those reported by DEV and MUKHERJEE (1986) and THOMPSON and co-workers (1982b). The NSI of rapeseed products were much higher in NaCl than in water and at pH 7.0; due to the salting effect of NaCl.

The water absorption of raw rapeseed meal was 313.2% similar to that reported by HORVÁTH and SENKÁLSZKY-ÁKOS (1990) with the same cultivars. Heated products had lower water absorption capacity than raw meal, it decreased also slightly by increasing the temperature. These results differ from those of SOSULSKI and co-workers (1976), on rapeseed products. NACZK and co-workers (1985) reported that the water absorption of commercial canola meal was 15–37% lower than that of the unheated canola meal, this is no doubt due to the exposure to high temperature during processing.

Rapeseed meals had fat absorption values ranging from 83–131%. Fat absorption decreased slightly by increasing heat treatment. These results are in agreement with those found by NACZK and co-workers (1985) and HORVÁTH (1988). Protein concentrate and isolate were superior in fat absorption than raw meal. The values of fat absorption of protein concentrate and isolate are markedly higher than those reported by THOMPSON and co-workers (1982a) and DEV and MUKHERJEE (1986).

The results on emulsification properties show that the emulsifying activity and emulsion stability of a raw meal is in agreement with those reported by HORVÁTH and SENKÁLSZKY-ÁKOS (1990) on the same cultivars of rapeseed. Emulsifying properties decreased gradually with increasing temperature. In general, all rapeseed products show higher values of emulsifying capacity than emulsifying activity and emulsion stability. Protein concentrate and isolate have low values of emulsification properties, although they have a high value of NSI. These results differ from those reported by DEV and MUKHERJEE (1986). They reported that emulsification properties of protein products are related to their soluble protein content. CHERRY and co-workers (1975) noted that the increases in emulsification properties observed were accompanied by decrease in amount of soluble protein.

Table 6

Functional properties of rapeseed products

Rapeseed products	pH of suspension in water	NSI in water (%)		NSI at pH 7.0 (%)		NSI in NaCl 1 mole (%)		Water[a] abs. (%)		Fat[a] abs. (%)		Emulsifying activity (%)		Emulsion stability (%)		Emulsifying capacity (cm^3 oil per g)	
		$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Raw meal	5.97	35.64	2.6	32.59	1.0	66.19	0.3	313.20	5.9	131.55	3.0	49.59	0.8	52.62	0.9	172	10.8
Toasted meal	5.62	22.31	1.4	21.65	0.8	38.82	0.4	183.24	8.2	83.57	4.9	8.34	0.8	7.04	1.0	84	7.6
Autoclaved meal, 1 h	5.41	13.03	0.8	13.21	0.5	25.19	2.1	192.89	9.6	109.36	4.6	41.40	1.7	21.08	1.5	147	10.3
Autoclaved meal, 1 1/2 h	5.21	7.55	0.6	7.54	0.3	10.18	2.4	180.35	8.1	105.52	3.4	31.29	0.5	10.90	1.2	140	8.0
Protein concentrate	5.15	7.96	0.6	56.84	1.3	56.66	0.4	101.17	6.2	248.58	6.9	18.08	1.6	4.30	0.2	80	10.0
Protein isolate	4.17	1.05	0.2	57.31	0.7	44.48	0.2	132.97	4.2	225.14	1.2	1.99	0.2	2.17	0.2	78	10.0

[a]Mean of values four determinations

Mean of values three determinations

3. Conclusions

The yield of protein isolate using single-step precipitation was higher in comparison to other reported recoveries of rapeseed isolate using a similar single-step precipitation (SOSULSKI & DABROWSKI, 1984).

Soaking the precipitate in sodium metabisulfite before drying yielded lighter coloured protein isolate as compared to washing the precipitate twice with 50% ethanol and once with distilled water (THOMPSON et al., 1982a).

Isolation of protein removed 95% of glucosinolates content similarly to those reported by SOSULSKI and DABROWSKI (1984), while the rate of reduction of phytic acid was much higher than those reported by BINZHOU and co-workers (1990).

Heat treatments led to decrease of all functional properties of rapeseed meals but still had satisfactory functional properties.

Protein isolate has better functional properties than those reported by DEV and MUKHERJEE (1986) except of emulsifying activity and emulsion stability.

The functional properties of protein concentrate were similar to those prepared by the same method with the exception of the emulsifying capacity which was lower than in the other samples.

On the basis of the functional properties of rapeseed products we draw the following conclusions:

- autoclaved meals and both protein isolate and concentrate could be used as extenders for meat protein;
- both protein isolate and concentrate could be added to some neutral beverages or foods.

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CHANGES OF SURFACE COLOUR OF THE FRUIT AND OF THE ANTHOCYANIN CONTENT OF SOUR CHERRIES DURING FROZEN STORAGE

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Three sour cherry cultivars were studied in two successive growing seasons. Changes in colour and in colouring matter content were tested during a one year storage and one of about 200 days. The changes during storage time were analyzed and the relations between colour indices and anthocyanin content were studied.

During frozen storage, the hue changes from red towards yellow. The red character mostly gets weaker and the yellow hue strengthens as a function of time. Both of them are of slight intensity and the latter is non-significant in case of either of the samples tested.

Colour of the samples become lighter in a slight degree. This change is significant up to the 200th day of the storage.

The chroma shows a slowly decreasing tendency.

The changes in anthocyanin content show different tendencies in the two successive seasons studied. In the first year, this content increases significantly with the length of storage time, in the second, a significant decrease can be observed. This difference could be ascribed to the fact, that in the second year, the starting of the experiment has been put to a date 24 days later, and there had to be in consequence a difference in the maturity of the fruits. In the less ripe fruits /1st year/, the biosynthesis of anthocyanins continues, according to the results found by several researchers. In case of more ripe fruits, this goes much slower or does not occur at all.

The correlations between the anthocyanin content and the various colour stimulus characters are not unambiguous. This can be attributed to the fact, that the colour stimulus depends not only on the anthocyanin content but also on physical and chemical interactions between pigments and other compounds, not even speaking from the optical effects of the physical state of the fruits. These effects are represented distinctively in the change of various colour stimulus characters.

Keywords: colour of sour cherry, anthocyanin of sour cherry, frozen sour cherry

The colour in our natural foods is produced by a combined visual effect of various coloured compounds included. In the formation of the colour of fruits, the flavonoids, the chlorophylls and the carotenoids are taking part. The red colour of sour cherries being object of our studies is produced primarily by the anthocyanins belonging to the group of flavonoids. This can produce beside red also blue and purple hues in our various kinds of plants (ARTHEY, 1981). The great diversity in colour of plants is the result of several anthocyanins being present simultaneously and of their changing proportion in the plant (GOMBKÖTŐ, 1973).

DARAVINGAS and CAIN (1968) found, testing fruit juices and model solutions, that the decrease of anthocyanin content can be ascribed to colour damages. pH value, temperature and oxygen are mostly responsible for the degradation of anthocyanins.

SHRIKHANDE (1976) gives a detailed review of anthocyanins as food colourants. Stability problems are dealt with, in connection with the chemical structure, and effects of pH, temperature, light, ascorbic acid, oxygen, metals, sugars and their products of decomposition, further of enzymes are studied.

Bibliographical review of stability problems is given by FRANCIS (1989), with consideration of the effects of pH-value, SO_2, ascorbic acid, metals, sugars, enzymes and temperature.

The colour changes of various processed fruits as a function of storage temperature were studied by ABERS and WROLSTAD (1979). They proved in their researches, that the red colour changes to brown above a temperature of 18 °C.

The processes producing colour changes in quick frozen foods are well known in many of the cases. The colour change can be the result of

- decolourization or transformation of natural colourants
- migration of colouring matters into the environmental medium
- formation of foreign colour from uncoloured components (BEKE, 1978).

WROLSTAD and co-workers (1970) studied the effect of pH in case of quick frozen strawberries. pH was found to be the unique objective factor giving a positive correlation to colour quality. It was found, that pH in the fruit has to be 3.51 or lower to arrive to an acceptable colour.

POLESELLO and co-workers (1986) observed, that the colour of blackberries, harvested in North Italy, changed to red in frozen storage. In a study where a storage of 6 months had been applied at a temperature of −20 °C, significant anthocyanin content increase has been observed by multi-variate regression analysis. This change depended on maturity and cultivar. A significant relation was found with the anthocyanin content between Hunter L-value and values $\sqrt{a^2+b^2}$.

An increase in anthocyanin content during frozen storage has been stated by KYZLINK and VIT (1971) for strawberries, by PIZZOCARO and co-workers (1979) for cherries and by CRIVELLI and ROCATI (1975) for raspberries. No unambiguous visual colour changes were here detected.

Relation between hue and anthocyanin content of undamaged sour cherries was studied by YEATMAN and co-workers (1961). There was found a close relation between the differences of absorbance as measured by spectrophotometer at wavelengths 540 and 612 nm and the anthocyanin contents. Similar problems were studied by DEKAZOS (1970), as well.

According to ALMÁSI (1977), the oxidation of anthocyanin and the polyphenol content are responsible for the change of red colour in sour cherries. The former causes decolourization, the latter browning.

As colour is a very important quality factor of coloured fruits and so of sour cherries, we tried to get find out what changes are generated in the hue and colouring matter content of sour cherries during frozen storage.

1. Materials and methods

1.1. Materials

As test materials, three sour cherry cultivars (Érdi bőtermő, Pándi 141, and Újfehértói fürtös) were used. The fruits were grown in Budaörs. After stemming, sorting and washing the sour cherries were placed in polyethylene pouches (~500 g per sample), then frozen in Lehel-Tyler freezing chamber at −20 °C. The storage temperature was the same. The studies were carried out in two successive growing seasons with the same varieties (1988/89 and 1989/90).

1.2. Methods

For the tests the samples were defrosted in a microwave oven. Twenty pieces each of thawed fruits being of room temperature have been used for colour measurements. Measuring was carried out with tristimulus colorimeter type Momcolor-D, the surface colour of fruit has been determined by reflectance method. As standard, the white standard No. 80-26-00 of the National Office of Measures, Hungary has been used where colour characteristics were $X_1 = 64.90$, $X_2 = 15.82$, $Y = 82.91$, $Z = 95.14$. Geometry of the measuring head 0°/45°, with 2° visual angle, CIE C illumination. Diaphragm diameter 10 mm.

For the determination of total anthocyanin content, the extinction of the solution was measured after an extraction with HCl–ethanol in a glass cuvette of 10 mm on 530 nm wavelength with a spectrophotometer type Spectromom. The total anthocyanin level was calculated from the absorbance (AUBERT, 1970).

Tests were made at the start of the study, then in the first test series (1988/89) during a storage period of one year at nine occasions, as a total. The second test series had to be finished due to technical problems after a storage of 196 days. During this period, in six occasions measurements were carried out. The test results were analyzed by mathematical statistical methods. As mostly linear relations were detected, the results of linear regression analysis are presented in our work.

2. Results

2.1. Change of colour as a function of the storage period

The colour stimulus characteristics in CIELAB system of the three sour cherry cultivars being stored in frozen state and tested in both years are presented in Table 1.

As to the colour change of the cultivar Érdi (Fig. 1), a shift toward the yellow hue during storage could be observed. In the first growing year, after the 272nd day, the direction of this change changed (in the 2nd year, the storage had to be ended due to technical problems). In both years, this was accompanied by a slight weakening of red character up to the 5th time (216th, 163rd day), and from this time on, a slight increase of red character could be observed.

Table 1

Colour stimulus characteristics and anthocyanin content of sourcherries during frozen storage
(mg per 1000 g dry matter)

Storage (day)	a*	b*	L*	C^*_{ab}	h^0_{ab}	Anthocyanin
1st year			Érdi			
0	20.75	3.71	23.02	21.10	10.14	541.4
76	17.85	5.22	22.26	18.62	16.48	1496.4
109	19.50	5.33	22.33	20.23	15.33	1298.5
188	21.46	5.35	23.21	22.14	13.95	1251.5
216	19.21	3.86	25.89	19.61	11.51	1705.1
242	18.23	5.61	24.15	19.09	17.36	1905.2
272	18.58	5.52	25.24	19.45	16.69	2016.2
336	20.36	4.11	24.29	20.78	11.51	1782.8
356	16.28	6.03	21.85	17.49	15.07	1877.4
1st year			Pándi			
0	28.80	4.76	19.85	29.21	9.40	1101.2
76	24.17	5.50	18.50	24.81	12.82	1400.6
111	22.54	4.78	20.96	23.06	12.00	1317.5
152	23.21	4.46	22.52	23.69	10.94	1139.4
209	20.99	4.37	23.33	21.49	12.13	1666.9
242	18.78	5.98	22.81	19.84	18.72	1674.5
272	18.96	4.37	22.32	19.49	12.89	1760.0
298	15.88	3.93	21.95	16.41	14.17	1714.7
355	20.98	7.01	19.39	22.19	19.47	1895.5

continued

Table 1 continued

	Storage (day)	a*	b*	L*	C^*_{ab}	h^0_{ab}	Anthocyanin
1st year				Újfehertói			
	0	30.52	5.77	20.05	31.09	10.58	792.8
	109	26.35	6.52	21.58	27.17	13.94	981.4
	188	27.68	5.64	21.73	28.28	11.70	855.6
	209	25.58	5.11	23.03	26.11	11.51	1325.0
	242	17.87	5.76	24.39	18.82	18.60	1185.5
	272	19.39	5.34	24.47	20.14	15.77	1626.5
	298	21.85	5.08	23.84	22.45	13.13	1309.6
	335	25.07	5.19	23.80	25.63	11.86	1393.8
	355	20.92	7.41	21.14	22.24	19.62	1226.9
2nd year				Érdi			
	0	12.42	6.21	18.79	14.02	27.77	1320.8
	35	11.63	5.16	20.09	12.79	24.18	1481.1
	70	13.65	6.77	21.96	15.27	26.35	813.1
	122	15.42	6.57	23.90	16.83	23.28	554.2
	163	11.73	6.60	25.21	13.50	29.40	577.1
	196	12.59	6.15	26.16	14.06	25.88	730.0
2nd year				Pándi			
	0	18.01	7.93	18.54	19.85	24.79	1039.3
	35	18.11	6.30	18.76	19.20	19.17	1503.3
	70	17.22	8.53	22.95	19.27	26.44	725.8
	122	18.97	8.42	24.54	20.77	23.98	789.1
	163	15.13	8.19	25.15	17.24	28.75	498.4
	196	17.66	7.59	26.51	19.24	23.28	726.5
2nd year				Újfehértói			
	0	17.23	7.32	19.91	18.74	22.83	1412.6
	35	15.31	6.79	20.98	16.80	24.04	1640.4
	70	15.72	8.39	24.17	17.86	28.27	880.5
	122	19.60	7.85	24.48	21.17	22.38	857.4
	163	18.17	7.93	24.69	19.87	23.83	734.1
	196	17.64	8.01	26.04	19.44	24.62	855.1
Limit values of s_x /standard deviation/		0.28 – 1.59	0.17 – 0.51	0.25 – 0.74	0.27 – 1.57	0.34 – 1.93	3.4 – 58.0

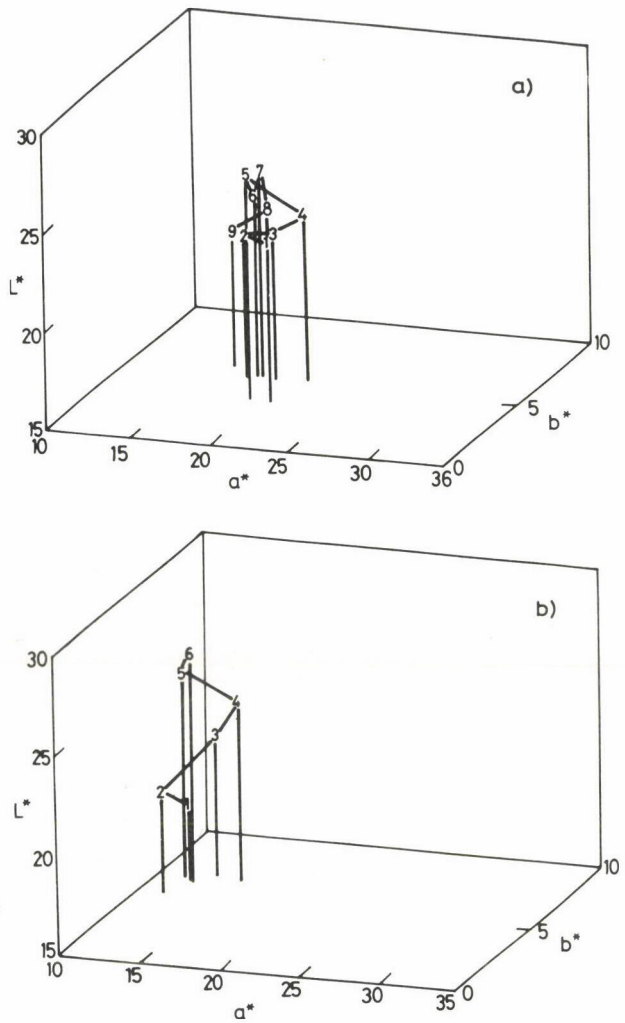

Fig. 1. Colour point in CIELAB space of frozen sour cherries (cultivar Érdi bőtermő) in various phases of storage. a: 1st year: 1 = The day of freezing, 2 = 76th day, 3 = 109th day, 4 = 188th day, 5 = 216th day, 6 = 242nd day, 7 = 272nd day, 8 = 336th day, 9 = 356th day; b: 2nd year: 1 = day of freezing, 2 = 35th day, 3 = 70th day, 4 = 122nd day, 5 = 163rd day, 6 = 196th day

Sour cherries from cultivar Pándi (Fig. 2) showed similar processes, but the decrease of the red hue was more intensive in the 1st year. This tendency changed after the 298th day.

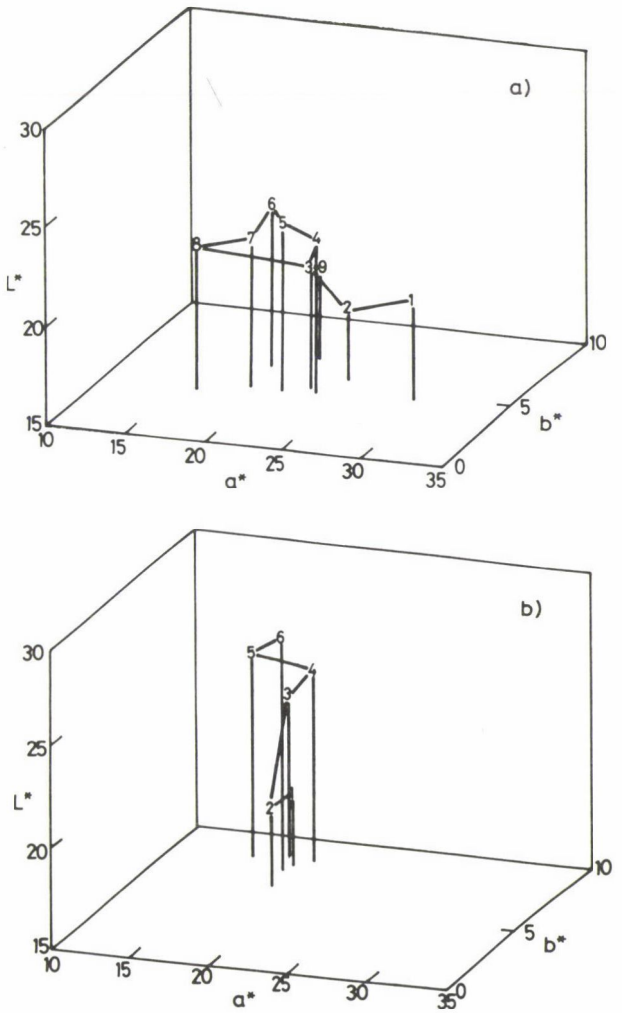

Fig. 2. Colour point in CIELAB space of frozen sour cherries (variety Pándi 141) in various phases of storage. a: 1st year: 1 = the day of freezing, 2 = 76th day, 3 = 111th day, 4 = 152nd day, 5 = 209th day, 6 = 242nd day, 7 = 272nd day, 8 = 298th day, 9 = 355th day; b: 2nd year: 1 = day of freezing, 2 = 35th day, 3 = 70th day, 4 = 122nd day, 5 = 163rd day, 6 = 196th day

In sour cherries cultivar Újfehértói (Fig. 3), a relatively more intense weakening of red character and a growing increase of yellow character could be observed up to the 242nd day, and at this point it changed in the opposite direction. The changes observed in the 2nd year were similar to those in the former two cultivars.

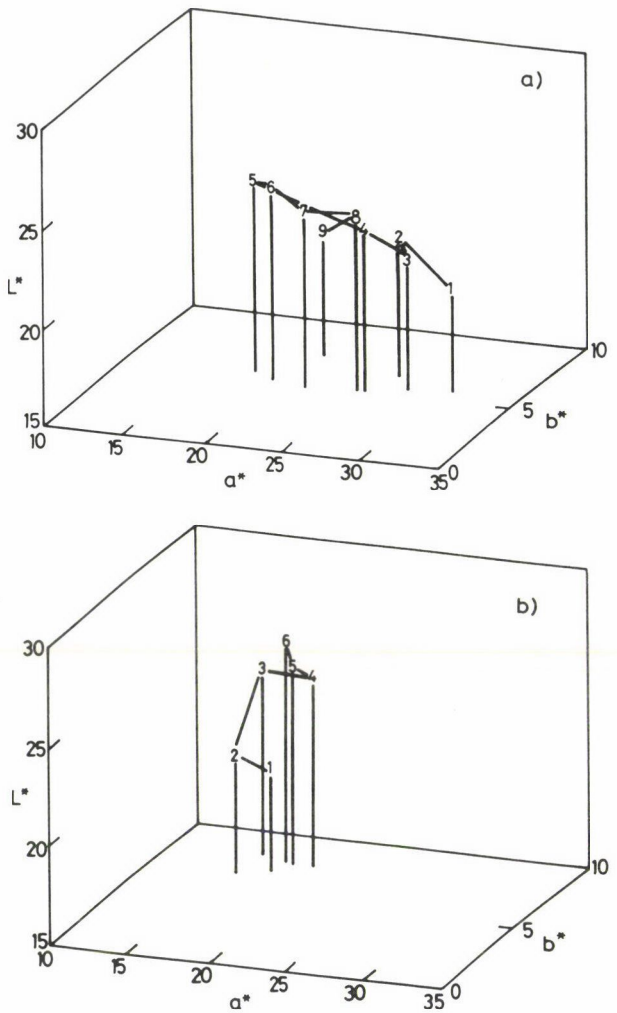

Fig. 3. Colour point in CIELAB space of frozen sour cherries (cultivar Újfehértói fürtös) in various phases of storage. a: 1st year: 1 = day of freezing, 2 = 109th day, 3 = 188th day, 4 = 209th day, 5 = 242nd day, 6 = 272nd day, 7 = 298th day, 8 = 355th day; b: 2nd year: 1 = day of freezing, 2 = 35th day, 3 = 70th day, 4 = 122nd day, 5 = 163rd day, 6 = 196th day

Evaluating the changes of the different colour stimulus characteristics as a function of storage time separately, for all six samples, an unambiguous change could be found only in a part of all cases. The red character (a*) decreases in most cases in function of time. This change was significant only for two samples and could be taken as being very slight. In significant cases, the slope of the straight line presenting the

change is of an order of 10^{-2} (a*/day). As an illustration, the change in time of the red character of the sample giving the closest relation has been presented in Fig. 4.

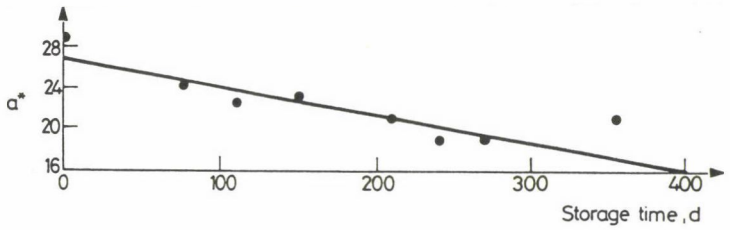

Fig. 4. Change of a* as a function of storage time (cultivar Pándi 141, 1st year) $r = -0.848$, $y = 26.814 - 0.027x$

Results of regression analysis are given in Table 2.

Table 2

Correlation coefficients (r) between colour characters and anthocyanin content and the storage time, based on linear regression analysis

Parameter	Érdi		Pándi		Újfehértói	
	r	P(%)	r	P(%)	r	P(%)
			1st year /n = 9/			
a*	−0.38	–	−0.85	99	−0.71	95
b*	0.34	–	0.25	–	0.01	–
L*	0.30	–	0.34	–	0.60	90
C^*_{ab}	−0.35	–	−0.82	99	−0.71	95
h^0_{ab}	0.18	–	0.75	98	0.51	–
	0.84	99	0.90	99	0.74	95
			2nd year /n = 6/			
a*	0.11	–	−0.37	–	0.53	–
b*	0.36	–	−0.26	–	0.57	–
L*	0.99	99.9	0.96	99	0.93	99
C^*_{ab}	0.15	–	−0.34	–	0.59	–
h^0_{ab}	0.08	–	0.32	–	0.01	–
	0.81	95	−0.70	90	−0.80	90

n: number of measurements
P: probability level

Yellow character (b*) increases for all but one sample but this is an unsignificant change.

The lightness factor (L*) rises parallel to storage period. The change is linear and highly significant in the tests made in the second growing season. In the first year, this change was not significant (Table 2). Taking a time period similar to that of the second year (about 200 days), the change here can be taken to be highly significant, too, with r values being between 0.82–0.93.

The rate of change is not too great, slope of the line being of 10^{-2} (L*/day) order. As an illustration, this relation has been presented in Fig. 5.

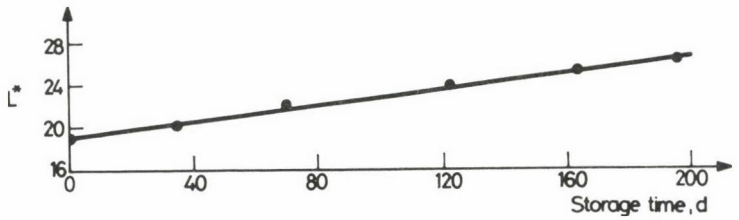

Fig. 5. Change of lightness factor (L*) as a function of storage time (cultivar Érdi bőtermő, 2nd year) $r = 0.996, y = 18.952 + 0.038x$

Chroma (C^*_{ab}) decreases generally during storage (Fig. 6.). This is significant only for two samples. The rate of change is slow, slope of the regression line being of 10^{-2} (C*/day) order.

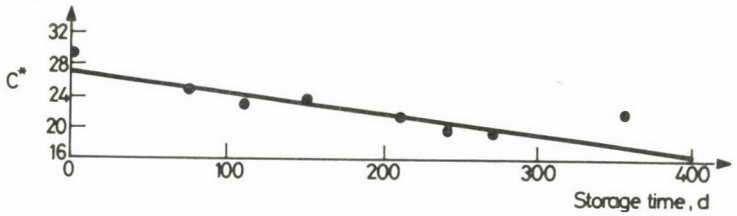

Fig. 6. Change of chroma (C^*_{ab}) as a function of storage time (cultivar Pándi 141, 1st year) $r = -0.820$, $y = 27.194 - 0.026x$

Colour of hue angle (h^0_{ab}) grows parallel to storage time showing the deplacement of the colour towards the yellow hue. This change is slight and can be taken as significant only for one sample.

2.2. Change of anthocyanin content as a function of storage time

Anthocyanin content shows significant and linear increase in function of storage time in the studies of the first year (Fig. 7). The slope of regression lines are 3.23, 2.24 and 1.76 mg%/day, respectively, showing the rate of changes for some cultivars.

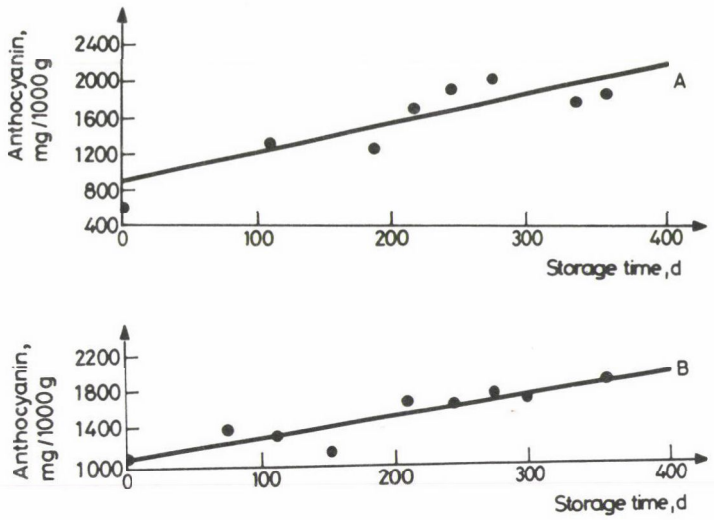

Fig. 7. Change of anthocyanin content as a function of storage time. A: cultivar Érdi bőtermő, 1st year $r = 0.837, y = 895.99 + 3.237x$; B: cultivar Pándi 141, 1st year, $r = 0.901, y = 1089.90 + 2.235x$

In the shorter storage studies in the second year, this change was in opposition to the former results. This suggested that in the various phases of storage time, different processes have taken place depending perhaps on the maturity degree of the fruit, as well.

2.3. Relation between colour stimulus characteristics and anthoyanin content

Based on our research results the relation between anthocyanin content and the various colour stimulus characteristics were tested. As the results of linear regression analysis were found to be best, these are presented in Table 3.

The relations are not considered to be unambiguous for either of the various colour characteristics. During the longer storage period (1st year), red hue (a*) was in close negative correlation with anthocyanin content (Fig. 8). In the second year, this relation was not determined.

A similar finding can be given for the chroma, as well. As the visually sensed colour can be described by more colour attributes, and the colorants exercise their effect on each of them, multivariate regression analysis was carried out. The results of a 3-variate regression analysis were given in Table 4.

Table 3

Linear regression analysis of the relation between anthocyanin content and colour characters in CIELAB system

Parameter	Érdi		Pándi		Újfehértói	
	r	P(%)	*r*	P(%)	*r*	P(%)
			1st year /n = 9/			
a^*	−0.60	90	−0.77	98	0.70	95
b^*	0.48	–	0.32	–	−0.32	–
L^*	0.39	–	0.18	–	0.80	99
C^*_{ab}	−0.59	90	−0.74	95	0.72	99
h^0_{ab}	0.55	–	0.75	98	0.32	–
			2nd year /n = 6/			
a^*	−0.51	–	0.55	–	0.58	–
b^*	−0.79	90	−0.83	95	−0.91	98
L^*	−0.86	95	−0.82	95	−0.90	98
C^*_{ab}	−0.59	–	0.34	–	−0.69	–
h^0_{ab}	−0.11	–	−0.88	95	−0.23	–

n: number of measurements

P: probability level

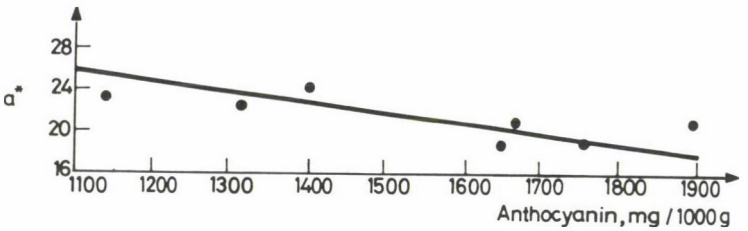

Fig. 8. Change of a^* in function of anthocyanin content (cultivar Pándi 141, 1st year) $r = -0.774$, $y = 36.884 - 0.010x$

Table 4

Three-variate regression analysis of relations between anthocyanin content and colour characters in CIELAB system of frozen sour cherries

Colour charcters		Érdi			Pándi			Újfehértói		
X_1	X_2	r	F	P(%)	r	F	P(%)	r	F	P(%)
				1st year (n=9)						
a*	b*	0.62	-	-	0.87	9.32	99	0.79	5.04	95
a*	L*	0.81	5.54	95	0.82	6.21	95	0.82	5.96	95
b*	L*	0.81	5.64	95	0.80	-	-	0.82	5.91	95
L*	C^*_{ab}	0.77	-	-	0.80	-	-	0.82	5.95	95
L*	h^0_{ab}	0.75	-	-	0.77	-	-	0.81	5.63	95
				2nd year (n=6)						
a*	b*	0.79	-	-	0.92	7.90	90	0.96	19.09	98
a*	L*	0.93	10.21	95	0.87	4.77	-	0.92	8.47	95
b*	L*	0.98	34.87	99	0.96	15.89	95	0.96	15.74	95
L*	C^*_{ab}	0.95	14.41	95	0.83	-	-	0.93	9.71	95
L*	h^0_{ab}	0.86	4.22	-	0.99	121.59	99	0.90	6.22	90

n: number of measurements
P: probablity level
F: result of *F*-test

In most cases, anthocyanin content showed significant relations to a*, L* and b*, L* values, except some seasons of cultivar Pándi. Relations between anthocyanin content and a*, L* , values were described by the following equations of similar form, in the 1st year Pándi (1), 2nd year Érdi (2). and Ujfehértói (3) samples:

1. anthocyanin = $4234.4 - 72.5a^* - 54.0L^*$
2. anthocyanin = $4673.6 - 103.3a^* - 107.0L^*$
3. anthocyanin = $4882.1 - 54.5a^* - 123.1L^*$.

The b*, L* relations of anthocyanin content of cultivars Érdi and Ujfehértói have given similar equations for the same seasons.

1st year: Érdi: anthocyanin = $-6097.2 + 425.1b^* + 234.3L^*$

Újfehértói: anthocyanin = $-2782.5 + 761.b^* + 155.8L^*$

2nd year: Érdi: anthocyanin = $5071.8 - 351.5b^* - 86.6L^*$

Újfehértói: anthocyanin = $5462.4 - 345.3b^* - 74.2L^*$.

The different equation forms in case of significant relations, and the different values of coefficients in case of equations of the same form show that these relations are dependent on seasons and on cultivars, as well.

Results of 4- and 5-variate regression analyses are presented in Table 5.

The 4-variate regression analysis showed in the tested cases – with the exception of the relation anthocyanin and a*, b*, C^*_{ab} – significant and close relations. Significancy level was about 90 percent even for some exceptions. In order to consider the cultivar's and season's effect mentioned above, the relations are mostly described by equations of different forms. Equations of matching form were found in the 2nd year between two cultivars.

Based on 5-variate analysis, significant relation between anthocyanin content and a*, b*, L*, C^*_{ab} colour stimulus values was found only in the tests made with Pándi cultivar in the second growing season (Table 5).

3. Conclusions

Based on frozen storage tests carried out in two successive years with three cultivars of sour cherries, the following could be stated:

A shift from red toward yellow was observed in the hue. The red character decreases in most cases and yellow character increases as a function of time. The change in both cases is slight and the latter is not significant for either of the samples.

The colour of samples become lighter in a very small degree. This change is significant up to about the 200th day.

Table 5

Four and 5-variate regression analysis of relations between anthocyanin content and colour characters in CIELAB system of frozen sour cherries

Colour characters				Érdi			Pándi			Újfehértói		
X_1	X_2	X_3	X_4	r	F	P(%)	r	F	P(%)	r	F	P(%)
1st year (n=9)												
a*	b*	L*	-	0.89	6.37	96	0.87	5.33	95	0.82	3.33	88
a*	b*	C^*_{ab}	-	0.66	-	-	0.88	5.58	95	0.79	-	-
a*	L*	C^*_{ab}	-	0.88	5.62	95	0.87	5.11	94	0.82	3.34	88
b*	L*	O^*_{ab}	-	0.89	6.41	96	0.87	5.37	95	0.82	3.33	88
h^0_{ab}	L*	C^*_{ab}	-	0.84	4.08	92	0.87	5.12	94	0.83	3.58	90
a*	b*	L*	C^*_{ab}	0.89	3.86	90	0.88	3.35	86	0.82	2.01	75
2nd year (n=6)												
a*	b*	L*	-	0.99	33.39	97	0.99	51.26	98	0.98	19.67	95
a*	b*	C^*_{ab}	-	0.86	1.84	-	0.97	10.75	90	0.96	8.62	90
a*	L*	C^*_{ab}	-	0.99	32.47	97	0.99	97.42	99	0.98	16.09	94
b*	L*	O^*_{ab}	-	0.99	33.61	97	0.99	47.13	98	0.98	19.38	95
h^0_{ab}	L*	C^*_{ab}	-	0.99	30.66	97	0.99	165.61	99	0.98	15.12	64
a*	b*	L*	C^*_{ab}	0.99	12.73	80	0.999	17037.4	99.5	0.99	10.15	78

n: number of measurementes
P: probability level
F: resault of *F*-test

The chroma shows a very slowly decreasing tendency. The change in anthocyanin content shows different tendencies in the two years tested. In the first season, it increases significantly with storage time, in the second, a significant decrease could be observed. This difference could be ascribed to the fact, that in the second year, the study was started 24 days later and in consequence a difference in maturity might have affected the results. In the less ripe fruits (1st year) during frozen storage the biosynthesis of anthocyanins continued slowly, similar to the findings published by KYZLINK and VIT (1971) for strawberries, by CRIVELLI and ROSATI (1975) for raspberries, and by PIZZOCARO and co-workers (1979) for cherries. In case of a more ripe fruit, this process is much slower or does not occur at all.

The relations between anthocyanin content and the various colour stimulus characters are not unambiguous. This could be ascribed to the fact, that the colour is not depending only on the anthocyanin content, but on physical and chemical interactions between pigments and other kinds of compounds, and on the optical effects of the physical state of the fruits. These effects are presented differently in the changes of the various colour values. The same has been observed by POLESELLO and co-workers (1986), in the case of blackberries, as well.

The joint relations of anthocyanin content and of several colour stimulus characteristics evaluated by multivariate analysis can be expressed in a number of cases by close correlations. However, an equation of similar form has been seen in case of different seasons and cultivars only in a few cases. This is indicative to the fact, that these relations are dependent on varietal differences and growing seasons, and – in this connection – with the degree of maturity, as well.

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STUDY OF AMINO ACID, ORGANIC ACID AND FREE SUGAR COMPOSITION OF NEW VALLEY DATES AND CERTAIN DATE PRODUCTS

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Two date cultivars grown in the New Valley Governorate, Egypt Saidy date (semi-dry variety) and Balady date (dry variety) were studied for their total amino acid, organic acid and free sugar compositions. One of the seedling dates was also investigated. Thin-layer chromatographic technique revealed that all the ten essential amino acids were present in variable levels in all studied samples. However, their levels gradually decreased in ripening stages of Saidy date. Balady date had highest levels of histidine, lysine and arginine, while seedling date fruits contained the highest levels of threonine, methionine, valine, leucine--isoleucine mixture, phenylalanine and tryptophan. Therefore, the latter variety could be reckoned as the highest in the total essential amino acids content. The same technique proved the presence of three organic acids (malic, citric and oxalic). Among them malic acid was the most predominant. On the other hand, sucrose, glucose and fructose were identified in the afore mentioned studied date samples. The data revealed that sucrose recorded the highest percentage in Balady variety. The glucose:fructose ratio ranged from 1.16-1.18 in all varieties except that in Balady variety, where it rated higher (1.25).

Keywords: date, amino acid, organic acid, sugar

Date was always considered as the most important desert crops all over the world due to its high nutritive and economic values. Dates contain essential nutrients, high sugar content as well as moderate percentages of protein, lipids, minerals and vitamins. Moreover, dates have high calorific, nutritive and medical value as well (YOUSSEF & RAMADAN, 1987). Chemical analysis among the more important date cultivars showed that total sugars in the flesh ranged from 47 to 85% according to cultivar and stage of fruit maturity (CAVELL, 1947; RAHMAN & ALI, 1955; MAKEL, 1967; HUSSEIN, 1970; RAMADAN, 1990). EL-SHAMERY and EL-DIEN (1988) evaluating the chemical composition of Yemeni and Egyptian dry dates found that moisture, carbohydrates, protein, ether extract, ash and total calories were 11.89% and 11.14%, 87.97% and 87.51%, 7.94% and 8.33%, 1.82% and 1.9%, 2.7% and 2.25% (on dry weight basis), and 361.05 and 364.63 calories (on wet weight basis); respectively. The contents of calcium, phosphorus, potassium, sulphur, sodium, chlorine, magnesium, iron, manganese, copper, zinc, cobalt and fluorine averaged from 133–207, 13–16, 833–894, 10–21, 5–16, 260–342, 56–60, 3.21–10–37, 5.14–5.86, 2.54–2.89, 0.79–1.82, 0.76–0.96 and 0.12–0.20 per 100 g respectively (on

dry weight basis) of four Iraqi dates cultivars (YOUSIF et al., 1982). Several workers reported that around 10–18 amino acids have been identified and quantitatively estimated in date of various cultivars (AL-RAWI et al., 1967; SALEM & HEGAZI, 1971; ABDUL HAFIZ et al., 1980; NOUR & MAGBOUL, 1985).

Dates had long been known in Arab countries, which produce 2098 thousand tons amounting to 70.5% of total world production. Egypt is one of the first largest date producer countries (545.000 tons) followed by Saudi Arabia (495.000 tons) and Iraq (350.000 tons) in 1988 (FAO, 1988).

Most of dates produced are consumed directly with little or no further processing. New products have been recently searched to establish outlets for the surplus dates and to present more assortments in consumption forms (EL-SHAARAWY et al., 1986).

Nitrogen content of dates is relatively small. Proteins and free amino acids are the main nitrogen containing components. Protein levels in dates range between 1.5–2.0% (AL-RAWI et al., 1967). AL-RAWI and co-workers (1967), SALEM and HEGAZI (1971), STERNKOPF and AMIN (1973), AUDA and co-workers (1976), ABDEL HAFIZ and co-workers (1980) and NOUR and MAGBOUL (1985) reported that around 10–18 amino acids have been identified and quantitatively estimated in date of various cultivars, RYGG (1946, 1948), EL-AZZOUNI and co-workers (1975), HUSSEIN and co-workers (1976) and EL-KASSAS and MAHMOUD (1984a,b) reported that the total acidity in dates was highest during the period of most rapid growth. It decreased during the later part of growing season and continuously decreased as the fruit ripened. Although organic acids contribute little to the nutritive value of dates, they do affect their flavour and quality (VANDERCOOK et al., 1979). To the author's knowledge no available reference concerning the identification of the organic acids in ripe dates were detected. Chemical analysis of the more important date cultivars showed that total sugars in the flesh ranged from 47 to 85% according to cultivars and stage of fruit maturity (ASHMAWI et al., 1955 and 1956; HUSSEIN ,1970; HUSSEIN & EL-ZEID, 1975; YOUSIF et al., 1982; EL-KASSAS & MAHMOUD, 1984a). COOK and FURR (1953) stated that the soft types contained low levels of sucrose, while the dry types had relatively higher levels with the semi-dry in between. SALEM & HEGAZI (1971) reported that the glucose:fructose ratio ranged from 0.92 to 1.25 in date fruits.

This investigation was carried out in an attempt to set an accurate comparison between Saidy and Balady dates as the most famous varieties with the unexplored, seedling date (or Manthour) and certain Saidy date products with respect to their total amino acid, organic acid and free sugar composition as well.

1. Materials and methods

1.1. Materials

This study was carried out on Balady dry date variety; Saidy semi-dry date variety and one unidentifiable seedling date. Five kg samples from each variety were obtained from Kharja Date Packing Factory, Kharja Oasis, New Valley Governorate, Upper Egypt in 1987 season. Samples were taken at random and transmitted to the laboratory for determination of amino acids, organic acids and free sugars. Pits were removed from date fruits, then the flesh was cut into small pieces and minced just before analysis. The studied date samples were taken in the forms as shown in Table 1.

Table 1

Date and date product samples

Abbreviation	Product
A	Saidy at Khalal stage (Yellow), mature
B	Saidy at 50 % Rutab (intermediate)
C	Saidy at Rutab stage (discoloured)
D	Over-ripe Saidy fruits
E	Seedling date (Manthour)
F	Balady dates (Tamr)
G	Fumigated Saidy fruits
H	Treated Saidy fruits (fumigated, washed, dried and selected) for export preparation
I	Treated Saidy fruits and prepared for local marketimg
J	Pressed Saidy fruit packaged as 1/2 and 1 kg in cellophane bags (Agwa)
K	Saidy fruit paste blocked in 1/2 and 1 kg cellophane bags
L	Saidy fruit paste mixed with sesame packaged in 1 kg cellophane bags
M	Pitted Saidy fruits stuffed with peanut
N	Pitted Saidy fruits stuffed with almond

1.2. Methods of analysis

1.2.1. Total acidity. Total acidity was determined as described in A.O.A.C. (1980), expressed as malic acid.

1.2.2. Protein content. Protein was estimated by multiplying the total nitrogen by appropriate factor, 6.25. Total nitrogen was determined using standard Kjeldahl method (A.O.A.C. 1980).

1.2.3. Essential amino acids. Essential amino acids (except tryptophan) were determined qualitatively and quantitatively by thin-layer chromatographic (TLC) technique as described by BLOCK and co-workers (1958); STAHL (1965) and EL-FISHAWY (1986). Tryptophan was determined colourimetrically using the method described by SASTRY and TUMMURU (1985), absorbance of the colour was measured at 500 nm.

1.2.4. Separation and identification of free sugars. Sugars were extracted using 70% neutral ethyl alcohol according to the method of KOZUKUE and co-workers (1965). Thereafter, sugars were fractionated into classes by TLC technique using silica gel as described by STAHL (1969) and TOUCHSTONE (1976). The isolated fractions were identified according to the R_f value of pure sugars on the same plates, and estimated colourimetrically as described by PESEZ and BARTOS (1974).

1.2.5. Separation and identification of organic acids. Organic acids were extracted from date samples as described by KOZUKUE and co-workers (1983). The organic acid extracts were evaporated in a rotatory evaporator at 30 °C then dissolved in 5 cm^3 70% ethanol. Thin-layer chromatographic plates coated with silica gel were used for separation of organic acids as described by BUCHBAUER (1972). The spray reagent of identification of organic acid fractions of date samples extract was used as outlined in STAHL (1969). The variation in relative levels organic acids were roughly estimated by visual comparison of area and intensity of colour of fractions corresponding to identified organic acids.

2. Results and discussion

2.1. Essential amino acid content

The results of the essential amino acids composition of the studied date samples are given in Table 2.

The identified essential amino acids (except tryptophan) in descending order of R_f values were the following: phenylalanine, leucine-isoleucine mixture, methionine, valine, threonine, arginine, histidine and lysine. Comparable R_f values were obtained by EL-FISHAWY (1986) upon fractionation of essential amino acids of cottonseed proteins, using the same chromatographic conditions.

Data given in Table 2 revealed that Saidy fruits at Khalal stage contained a high level of leucine-isoleucine mixture (186.88 mg per 100 g) and lysine (149.50 mg per 100 g) followed by arginine (136.39 mg per 100 g); threonine (108.20 mg per 100 g); valine and phenylalanine at almost equal levels (92.13 mg per 100 g); methionine (82.00 mg per 100 g); tryptophan (75.63 mg per 100 g); and histidine (68.20 mg per 100 g). While, in the overripe stage, Saidy fruits contained high levels

Table 2

Mean values of essential amino acids in mg per 100g on dry weight basis

Date samples	Lysine	Histidine	Arginine	Threonine	Valine	Methio-nine	Leucine + isoleu-cine	Phenyl-alanine	Tryptophan	Total E.A.A.
A	149.50	68.20	136.39	108.20	92.13	82.00	186.80	92.13	75.63	991.06
B	139.96	55.97	105.95	93.96	79.97	70.98	167.95	83.98	66.88	865.60
C	106.00	46.61	105.88	92.15	79.91	70.76	153.99	71.07	64.38	790.75
D	100.20	30.19	80.00	89.25	75.17	62.97	150.31	60.13	64.38	712.60
E	144.80	117.07	134.16	124.81	110.95	93.21	266.42	91.60	100.00	1183.02
F	171.88	124.14	152.00	105.04	95.49	76.40	257.82	85.94	97.50	1166.21
G	98.27	28.70	78.27	88.40	68.99	58.85	147.84	59.14	58.13	686.59
H	91.07	26.07	70.40	78.31	70.83	55.96	141.67	60.72	53.75	648.78
I	95.17	28.29	74.70	88.70	62.82	59.29	136.11	51.41	55.63	652.12
J	97.47	26.80	78.29	87.47	72.64	54.98	140.78	52.49	55.00	665.92
K	94.50	27.81	78.45	87.81	74.17	54.52	149.08	53.87	55.63	675.84
L	136.60	105.99	127.19	129.80	115.40	104.80	258.99	103.60	93.75	1176.12
M	141.73	122.16	182.50	131.87	119.30	121.44	272.45	106.86	100.00	1298.40
N	143.01	143.22	184.30	132.79	132.15	140.36	273.70	111.93	105.00	1366.46

Data are the mean value of 3 determinations

Abbreviations for symbols A to N of date samples are given in Table 1

E.A.A.: Essential amino acids

of leucine – isoleucine mixture (150.31 mg per 100 g) and lysine (100.20 mg per 100 g) followed by threonine (89.25 mg per 100 g), arginine (80.00 mg per 100 g), valine (75.17 mg per 100 g), tryptophan (64.38 mg per 100 g), methionine (62.97 mg per 100 g), phenylalanine (60.13 mg per 100 g) and histidine (30.19 mg per 100 g), on dry weight basis.

The data presented in Table 2 show that the essential amino acids levels were considerably different in Saidy variety when ripe or over-ripe, respectively. Conclusively, the essential amino acids decreased gradually after the Khalal stage.

From Table 2 it is obvious that the essential amino acid contents of Saidy fruits at ripe stage were higher than those reported by AL-RAWI and co-workers (1967) for the three Iraqi, semi-dry dates (Hallawi, Khadrawi and Sayer). Moreover, Auda and co-workers (1976) stated that the Iraqi dates (Khastwi, Khadrawi and Zahdi) contained lower levels of the essential amino acids except for valine, leucine – isoleucine mixture and phenylalanine compared with the Saidy variety.

Moreover, the data revealed that the treated and processed Saidy fruit contained high levels of leucine – isoleucine mixture (136.11 – 273.70) and arginine (70.40 – 184.30) followed by histidine (26.07 – 143.22), lysine (90.07 – 143.01), methionine (54.52 – 140.36), threonine (78.31 – 132.79), valine (62.82 – 132.15), phenylalanine (51.41 – 11.93) and tryptophan (53.75 – 105.00) mg per 100 g. On the other hand, the levels of individual essential amino acid and the total essential amino acids of the stuffed Saidy fruits were the highest among other products. This could be attributed to the stuffing matter and its rather high protein content.

Comparison of the three varieties analysed regarding their essential amino acid composition, indicated marked differences in the quantities of individual and total essential amino acids, this seemed to be correlated with their respective protein contents. Balady variety had higher levels of lysine (171.88 mg), arginine (152.00 mg), and histidine (124.12 mg) per 100 g, than Manthour followed by Saidy.

Table 3

Analysis of variance of the data given in Table 2

		M S				
S O V	D F	Lysine	Histidine	Arginine	Threorine	Valine
Treat	13	2123.8**	5961.18**	4780.68**	1115.3**	1446.59**
Error	26	38.05	1.96	4.34	2.94	2.56

** Highly significant at $P \geqslant 0.01$ probability level

Manthour fruits contained higher levels of leucine-isoleucine mixture 266.42 mg; threonine 124.81 mg; valine 110.95 mg; tryptophan 100.00 mg; methionine 93.21 mg; phenylalanine 91.60 mg per 100 g; and total essential amino acids than cultivar Balady followed by Saidy variety. The levels of essential amino acids in Balady variety are in line with those reported by SALEM and HEGAZI (1971). On the other hand, the three studied dates had lower levels of essential amino acids than the mean concentrations in 15 soft date fruit samples, grown in Saudi Arabia as reported by ABDEL-HAFIZ and co-workers (1980).

In Table 3 the analyses of variance data of essential amino acids are summarized in the studied samples. It should be noted that there were highly significant differences among the three varieties as well as between treated and processed Saidy dates.

2.2. TLC-separation and identification of organic acids

The acidity of dates usually decreased with ripening. A high pH value is a characteristic of dates of high quality (RYGG, 1948).

The results of TLC analysis of organic acids in date samples studied are represented in Table 4. It is apparent that there were three identified organic acids namely: malic, citric and oxalic. These results indicated that malic acid was the main organic acid in all samples followed by very low level of citric and oxalic acid. Such results are in partial agreement with data previously reported (VANDERCOOK et al., 1979).

				F	
Methionine	Leucine + iso-leucine	Phenyl-alanine	Tryptophan	0.05	0.01
2155.45**	10004.25**	1401.62**	1196.52**	3.37	5.53
3.42	5.41	3.06	3.29	2.69	4.16

Table 4

Identification and qulitative evaluation of organic acids

Date samples	Presentation of organic acid					Total acidity as malic acid (%)
	Malic	Citric	Tartaric	Oxalic	Succinic	
A	+ + + +	+	-	+	-	0.142
B	+ + + +	+	-	+	-	0.135
C	+ + + +	+	-	+	-	0.127
D	+ + + +	+	-	+	-	0.121
E	+ + + +	+	-	+	-	0.183
F	+ + + +	+	-	+	-	0.152
G	+ + + +	+	-	+	-	0.125
H	+ + + +	+	-	+	-	0.125
I	+ + + +	+	-	+	-	0.129
J	+ + + +	+	-	+	-	0.122
K	+ + + +	+	-	+	-	0.117
L	+ + + +	+	-	+	-	0.117
M	+ + + +	+	-	+	-	0.118
N	+ + + +	+	-	+	-	0.116

Abbreviations for symbols A to N of date samples are given in Table 1

+ + + + : Very high level

\+ : Very small level

\- : Absent

In an over-all look at the above mentioned results of total acidity values in the date samples studied, it could be seen that malic acid was the most effective acid in determining the acidity values, especially when no contribution from date fermentation occurred.

2.3. TLC-identification and quantitative evaluation of free sugars

Three sugars namely: glucose, fructose and sucrose were separated from the date samples studied, however in different amounts. The area of the glucose spot is larger than that of fructose, but sucrose has a small area in all samples, except for Balady fruit recording the largest area for sucrose. These results are in partial agreement with that obtained for Zahdi Khadrawi and Berbin Iraqi dates by AL-DAWODY and co-workers (1967) and for SAMANI and ZAGHLOUL as reported by MANSOUR (1974).

Table 5

The percentage of individual sugars in the total sugars content

Date samples	Sugars /%/			Glucose:fructose ratio
	Glucose	Fructose	Sucrose	
A	50.83	43.06	6.11	1.18
B	50.60	43.01	6.39	1.18
C	50.58	43.04	6.38	1.18
D	51.13	43.33	5.55	1.18
E	50.97	43.57	5.46	1.17
F	26.32	21.05	52.63	1.25
G	50.88	43.68	5.44	1.17
H	51.33	44.25	4.43	1.16
I	51.66	43.73	4.61	1.18
J	52.15	44.64	3.22	1.17
K	52.25	44.81	2.94	1.17
L	51.79	44.64	3.60	1.16
M	51.71	44.36	3.94	1.17
N	51.70	44.53	3.77	1.16

Abbreviations for symbols A to N of date samples, are given in Table 1

The results given in Table 4, indicate that the percentage of glucose, fructose and sucrose varied from 50.83, 43.06 and 6.11% in the Khalal stage of Saidy dates. Sucrose slightly increased from Khalal to Rutab stage, while it decreased to 5.55% in over-ripe fruits. The glucose:fructose ratio of Saidy fruits was 1.18 during ripening and varied from 1.16 to 1.18 for treated and processed fruits being 1.17 and 1.25 for seedling and Balady dates, respectively.

These findings are in a reasonable agreement with those reported by SALEM and HEGAZI (1971), who found that glucose:fructose ratio in Egyptian dry date was 1.25. Meanwhile, HUSSEIN and EL-ZEID (1975) stated that the glucose:fructose ratio in Saudi date (Khalas) was 1.19. YOUSIF and co-workers (1982) found that the ratio of glucose:fructose was 1.17 in Hallawi, Sayer and Khadrawi, while it was 0.83 for Zahdi data. The date revealed that the Balady variety had the highest sucrose content (Table 5).

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DETERMINATION OF THE MOLECULAR MASS OF POLYGALACTURONIC ACID

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A study has been made for the determination of the molecular mass of polygalacturonic acid in aqueous solution (0.005 mol lithium-oxalate, 0.05 mol lithium-phosphate, pH = 7.3).

Starting out from natural (citrus) pectin, we produced, by heat degradation and alkaline hydrolysis, finally fractionation with iso-propanol, standard sodium salt of polygalacturonic acid fractions, the molecular masses of which were determined by light scattering measurements.

With calibrated sodium polygalacturonate salt fractions, we developed a technology for the determination of viscosimetric molecular masses, in which the Mark-Houwink equation is:

$$[\eta] = 4.368\ 10^{-7}\ M^{1.8737}$$

We conducted gel-chromatographic studies on a micro-column with calibrated sodium polygalacturonate salt samples.

Chromatograms were evaluated with the help of eluent volumes belonging to the peak height.

By the above methods, we have measured the molecular mass of polygalacturonate samples originating from commercial pectins.

Keywords: gel-chromatography, molecular mass, polygalacturonic acid, viscosimetry.

Intensive soil and plant cultivation – relating to a record quantity of yield – widespread in agriculture, closed intensive livestock breeding, and last but not least, the purification, refinement and often inadequate kitchen technical processing of our foods result in the lack of essential nutritive metal ions, such as magnesium, iron, zinc, copper, etc. We suggest (LAKATOS et al., 1980), that human and animal organisms should be orally administered deficient nutritive metal ions, in the form of metal polygalacturonates by fortification of foods and feeds with nutritive metal ions, since metal polygalacturonates contain metal ions in a very similar chemical bond to that of some of our nutriments, and are well miscible with them. Metals are excellently absorbed with metal polygalacturonates in living organisms and are well utilizable, too, without any harmful side effect. In the form of polygalacturonates, metal ions do not catalyze the degradation of vitamins sensitive to oxidation, and do not deteriorate organoleptic properties of our foods.

In order to provide a solution for the problem outlined above, we developed a technology using commercial pectin (polygalacturonic acid-methyl ester) as a starting substance, for the production of metal polygalacturonates (LAKATOS et al., 1980).

In our technology, we submitted pectin (*P*) originating from fruits, such as citrus, apple, etc., – by applying sodium hydroxide – to alkaline ester hydrolysis, when sodium salt of polygalacturonic acid (NaPGA) is formed. Simultaneously with alkaline hydrolysis, polygalacturonic acid (PGA) macromolecules suffer, according to beta-elimination mechanism, a chain-breaking, too, i.e. depolymerize (BEMILLER, 1986). Following ester hydrolysis, pure PGA was separated by precipitation with hydrochloric acid, washing with diluted hydrochloric acid and separation in a centrifuge, and it was further used for the production of metal polygalacturonates.

Biological effectivity of these products largely depends on the molecular mass of PGA macromolecules constituting the sample itself. Biologically the most effective macromolecules are that of 3500 – 25000 D molecular mass (LAKATOS et al., 1980).

In order to obtain a biologically most effective molecular size, reaction conditions in the alkaline hydrolysis of *P* have to be optimally chosen. Therefore, for the determination of optimal reaction conditions, it is of key importance to control, from time to time, changes in molecular size during the alkaline hydrolysis of *P*.

References dealing with the chemistry of acidic polysaccharides has not provided a reliable method until today, for the determination of the sizes of PGA molecules, which can be accomplished with quick, precise and relatively simple instruments, and can be applied as a routine.

There is a great number of publications on the study of macromolecular properties of *P* substances used as a standard material: OWENS and co-workers (1946); GLIKMAN and ORLOV (1950); FRITSCHE and co-workers (1977); BERTH and co-workers (1977) gave account of examinations made by viscosimetric and osmometric methods, FISCHMAN and co-workers (1984); ANGER and BERTH (1986) reported on examinations by gel-chromatographic methods.

Due to alkaline ester hydrolysis, the form and structure of *P* molecules essentially change, free carboxyl group content of *P* increases, and at the same time, *P* degrades. Therefore, previous methods and calculations applied in the study of macromolecular properties of *P* cannot be directly used in the examination and qualifications of PGA substances.

To provide a solution for the outlined problem, in the framework of an overall study, we invented to develop – by using PGA standard substances calibrated by an independent physical method – an easily applicable, quick and reproducible method for the laboratory and pilot-plant sample study of macromolecular properties of PGA substances. For the accomplishment of these studies, combination of light scattering method with viscosimetry and gel-permeation chromatography seems to be the most suitable one.

1. Materials and methods

We applied, in our examinations, Genu x 6866, Grindsted 1400, Herbstreith NV-50 T P preparations, Serva PGA, as well as lithiumhydroxide, phosphoric acid, oxalic acid and hydrochloric acid, in A.R. quality, produced by Reanal Pharmaceutical Works.

Viscosimetric studies were conducted on a temperated micro-Ubbelode viscosimeter prepared at the Central Research Institute for Chemistry of the Hungarian Academy of Sciences.

Light scattering measurements were made on SOFICA instruments.

Gel-chroamtographic studies were carried out on a KhZh-1309 type Laser micro-gel chromatograph developed by the Science and Technology Corporation of the Academy of Sciences of the U.S.S.R., Leningrad.

All studies were conducted in a pH = 7.3; 0.005 mol l^{-1} lithium oxalate solution containing 0.05 mol l^{-1} lithium phosphate (LiPB) buffer solution.

1.1. Production of sodium polygalacturonate (NaPGA) fractions

We wished to produce NaPGA fractions of different average molecular masses from commercial *P*. The isolated *P*, i.e. methyl ester of polygalacturonic acid degrades – in an aqueous solution, in neutral and alkaline pH range –, according to beta-elimination mechanism catalyzed by OH-ions. Parallel with the increase of temperature, the speed of degradation significantly increases (MERRIL & WEAKS, 1945; ALBERSHEIM et al., 1960). By using these phenomena and following this, by applying the method of solvent precipitation, we intended to produce PGA fractions of different molecular masses.

Fifteen g commercial (Grindsted 1400) *P* was moistened, in a 2 dm^3 Erlenmayer flask, with 15 cm^3 ethanol, and was suspended, in 1500 cm^3 distilled water, with the application of an Ultraturrax type homogenisator (IKA-Janke Kunkel, Germany). Following this, with a required part of previously measured 1 mol l^{-1} sodium hydroxide solution of a volume of 90 cm^3, the pH value of the suspension was set to value of 7.0. pH measurement was performed with the application of a glass electrode. *P* suspension dissolved. The flask containing the neutral *P* solution was placed, for a period of one hour, into preheated incubators of 0 °C, 45 °C, 75 °C and 90 °C temperatures, and while adding the further parts of 1 mol l^{-1} sodium hydroxide solution of a volume of 90 cm^3, in order to maintain the pH value of the solution at 7. As a result of heat treatment, *P* molecules degraded as a function of temperature. Following this, the temperature of *P* solutions was changed for 25 °C, and the residual part of the previously measured 1 mol sodium hydroxide solution of a volume of 90 cm^3 was added to the *P* solution, and alkaline hydrolysis

was in process for one hour. By adding hydrochloric acid, the alkaline ester hydrolysis was stopped at a pH value of 7. The concentration of sodium ions was increased to 0.156 mol l^{-1}, by adding the solution of 3.81 g sodium chloride dissolved in 30 cm^3 distilled water, to the hydrolysate, during intensive mixing. Finally, the volume of the hydrolysate was supplemented with distilled water to a volume of 1650 cm^3.

NaPGA-containing solutions prepared as above were placed, for a period of 16 hours, into a refrigerator of a temperature of +4 °C when the non-degraded insoluble fraction of PGA separated out, in the form of precipitate, at a concentration of 0.156 mol sodium ion (1st fraction). The separated NaPGA-salt precipitate was isolated then in a coolable centrifuge (Janetzky K-70 6x600 cm^3 angle rotor, 3200 r.p.m. 30 min). From the separated supernatant, the degraded NaPGA fraction was isolated, by adding iso-propanol, in 1:10 volume proportion. Iso--propanol was added to the supernatant by dropping, during intensive mixing. Following this, the suspension was placed, for a period of 16 h into a refrigerator of a temperature of +4 °C. The isolated, degraded NaPGA precipitate (2nd fraction) was separated by centrifugation, as described above. Finally, this NaPGA precipitate was washed three times by suspending in the mixture of 10 portions of 0.12 mol sodium chloride and 1 portion of iso-propanol, then sedimentated by centrifugation and freeze dried. The contaminating sodium chloride originating from the wash liquor was washed with aqueous ethanol of 70% (w/w), and the preparation was dried in a vacuum drying oven, at a temperature of 40 °C.

The obtained fractions were marked, according to the temperature of pre--incubation, by the indications 0 °C-I-2; 45 °C-II-2; 75 °C-III-2; 90 °C-IV-2.

In our experiments, we also used NaPGA-salt prepared from (PGA) of a molecular mass of 30 000 D, marketed by the German SERVA Company, which was produced in an aqueous medium by neutralization with a sodium hydroxide solution up to a pH value of 7.3, and then freeze dried (SERVA NaPGA).

1.2. Production of experimental PGA samples

Metal polygalacturonate preparations listed in the patent of LAKATOS and co-workers (1980), are produced with the utilization of commercial *P*, as a raw material, originating from fruits, such as citrus, apple. Alkaline polygalacturonate produced by the alkaline ester hydrolysis of *P* is an intermediate of the production process, the macromolecular size of which influences biological effectivity of the preparation. For the plant control of PGA intermediate products, the determination of molecular mass is inevitable.

Four g pectin was suspended in 124 cm^3 distilled water (at 25 °C) and, following in the adding of 4 cm^3 4 mol l^{-1} sodium-hydroxide solution, ester hydrolysis was in process for one hour. PGA was precipitated with 21 cm^3, 1.2 mol l^{-1}

hydrochloride solution and, after 30 min, PGA-gel was separated by pressing on a filter linen. PGA-gel was twice suspended in 80 cm^3 0.1 mol l^{-1} hydrochloride solution for washing and separated by pressing on a filter linen, as described above, and was dried at room temperature. Humidity content of the preparation was determined by drying at 105 °C and its acid capacity was determined by titration with sodium hydroxide solution.

1.3. Determination of the molecular mass of NaPGA fractions by light scattering measurement

Mass average molecular mass of the produced NaPGA-salt fractions was determined by light scattering measurement method (SOFICA light scattering instrument). Measurements were conducted in LiPB solution. Results obtained by us are shown in Table 1.

1.4. Determination of molecular mass of NaPGA fractions by viscosimetric method

Viscosimetric study of NaPGA fractions calibrated by light scattering measurement method, as described in Para. 1.3. was conducted by applying LiPB solution. Viscosity of the solutions was determined in a viscosimeter thermostated with a micro-sized Ubbelode type water mantle (outflow time (t_o) of LiPB solution at 25 °C was 41.76 sec).

With NaPGA fraction, a solution of 0.4% was prepared, which was then filtered on a Sartorius SM 11306 filter of a 0.45 μ pore size.

Into the viscosimeter, a filtered solution of a volume of 3 cm^3 was introduced, the outflow time (t_m) of which was measured at a temperature of 25 °C. Following this, exact dilutions were prepared by introducing further parts of the buffer solution:

	added buffer	total volume	dilution
1.	+1 cm^3	4 cm^3	1.33
2.	+1 cm^3	5 cm^3	1.66
3.	+1 cm^3	6 cm^3	2.00
4.	+2 cm^3	8 cm^3	2.66
5.	+2 cm^3	10 cm^3	4.00

For each dilution, we determined outflow times (t_m). From these samples, a 0.4% solution was prepared in the eluent LiPB solution, to which we added sodium hydroxide, in the form of 1.0 mol solution, according to the value of acid capacity. So, pH value of the solution was set back to the required value of 7.3.

Values of intrinsic viscosity, as well as that of molecular masses determined by calculations made according to Para. 2.2. are shown in Table 3.

1.5. Elaboration of the gel-chromatographic study of NaPGA fractions

There are several publications on the gel-chromatographic (GPC) study of *P* and PGA samples with different methyl esterification degree produced by alkaline ester hydrolysis (ANGER & BERTH 1986; FISCHMAN et al., 1984), however, there has not been any GPC method published until today, for the determination of the molecular mass of PGA samples without methyl ester content produced by 1-3 method.

We endeavoured in our studies, to develop, by using NaPGA standard fractions produced by us in Para. 1.1., and calibrated by light scattering measurement, a quickly reproducible GPC method, for the determination of the molecular mass of laboratory and pilot-plant substances.

We conducted our GPC experiments with the application of KhZh-1309 type micro gel chromatograph, comprising a laser refractometric detector. For analytical gel-column, we applied a Teflon micro-column of 0.5 x 300 mm, filled with the fraction of "GMA"-gel (glycidil-polymetacrilate) of a grain size of 5 μ, developed by the Research Institute of Macromolecular Chemistry of the Czechoslovak Academy of Sciences, Prague. For eluent, we applied LiPB solution, also applied in the light scattering measurement method, as well as in viscosimetric measurements. During our information studies, we stated that a further increase of the concentration of components constituting the buffer solution did not modify eluent times in GPC studies.

In our GPC experiments, we prepared, in the LiPB solution used as an eluent, a 0.2% sample solution from the NaPGA standard fractions (produced by Para. 1.1.), eventually from PGA samples (produced by Para. 1. 2.) or available for us in the form of free acid (PGA produced by SERVA-firm), which was then filtered on a Sartorius SM 11306 type filter of a pore size of 0.45 μ. During our chromatographic study, we introduced it into the micro-column in a volume of 0.06 μl, in which we established an eluent flowing speed of 5 $\mu l\ min^{-1}$.

The results of GPC experiments are shown in Fig. 1 (NaPGA standards and SERVA sample) and Fig. 2 (PGA samples). The GPC eluent curve was evaluated point by point, on the basis of the correlation of the eluent volume and the dependent concentration. We determined molecular masses belonging to each eluent volume (M_w), as well as the value of signal height proportional to the concentration on the chromatogram (h_i). By applying the following correlations:

$$\overline{M}_w = \frac{\sum h_i M_{wi}}{h_i}$$

$$\overline{M}_n = \frac{\sum h_i}{\sum \frac{h_i}{M_{wi}}}$$

we calculated the mass average $\overline{M}_w$ and number average $\overline{M}_n$ molecular masses.

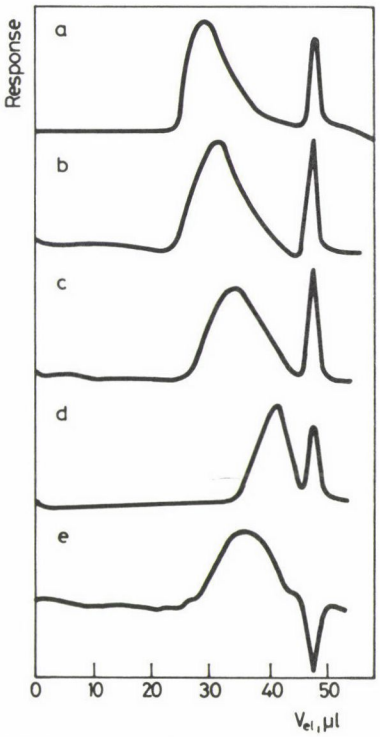

Fig. 1. GPC profile of NaPGA standards and Serva PGS

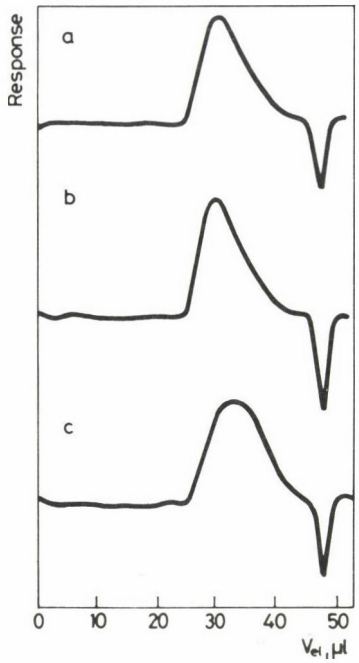

Fig. 2. GPC profile of experimental PGA samples

2. Results and discussion

2.1. Light scattering studies

The molecular mass of NaPGA fractions prepared by Para. 1. 1. and SERVA sample was determined by light scattering method (see Para. 1. 3.) in LiPB solution.

The mass average molecular mass values can be seen in Table 1

Table 1

The mass avarage molecular mass values of NaPGA fractions

NaPGA fraction	Temperature of degradation	Mass average (molecular mass M_WD)
I-2	0 °C	$57 \pm 5 \times 10^3$
II-2	45 °C	$35 \pm 5 \times 10^3$
III-2	75 °C	$30 \pm 5 \times 10^3$
IV-2	95 °C	$13 \pm 5 \times 10^3$
SERVA NaGPA	–	$25 \pm 5 \times 10^3$

2.2. Viscosimetric studies

In our viscosimetric measurements (see Para. 1.4.), calculations were made on the basis of effluent times.

The relative viscosity values (η_{rel}) were determined according to the equation:

$$\eta_{rel} = t_m/t_0 \text{ and } \eta_{spec} = \eta_{rel}^{-1}$$

We also calculated the value of specific viscosity and the number of viscosity (η_{spec}/C_i), which is the quotient of specific viscosity and concentration of the measured solution. C_i is the concentration of NaPGA, expressed in gcm^{-3}.

Finally we plotted values of η_{spec}/C_i, in the function of C_i, and by regression calculation, we determined the value of intrinsic viscosity [η]

$$[\eta] = \lim_{C_i \to 0} (\eta_{spec}/C_i)$$

The determined values of intrinsic viscosity are shown in Table 2.

Table 2

Values of intrinsic viscosity

Fraction	$\bar{M}_W \times 10^{-4}$	[η]	log M_W	log[η]	V_{el} µl average
I-2	5.7	335.95	4.755	2.526	28.84
II-2	3.5	156.40	4.544	2.194	31.63
III-2	3.0	102.20	4.477	2.009	34.22
IV-2	1.3	21.45	4.113	1.331	41.16
SERVA NaPGA	2.5	80.25	4.397	1.904	36.04

Knowing the mass average molecular mass ($\bar{M}_W$), determined by light scattering measurement, and the value of intrinsic viscosity [η] of each NaPGA fraction, which were determined in the same buffer solutions, we could calculate constants of the well-known Mark-Houwink equation.

$$[\eta] = K M^{-\alpha}$$
$$\log [\eta] = \log K + \alpha \log M$$

log [η] versus log $\bar{M}$ values indicated in Table 2 were plotted in Fig. 3, and the line describing the Mark-Houwink equation was determined by regression calculation.

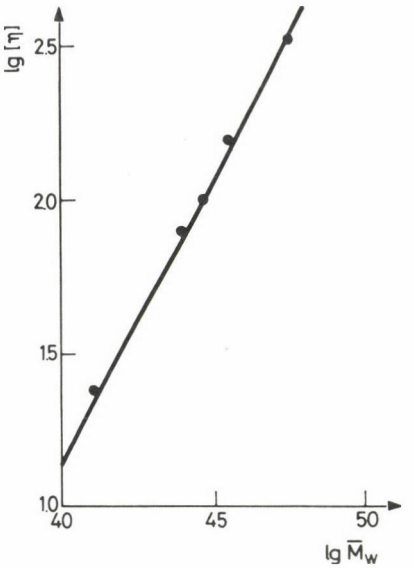

Fig. 3. Mark-Houwink plot for NaPGA standards

As a result of the calculations, we obtained the following correlations:

$$\log [\eta] = -6.3596 + 1.8737 \log \overline{M}$$

$$[\eta] = 4.368 \times 10^{-7} M^{1.8737}$$

$$(r = 0.997)$$

2.3. GPC studies

During the GPC study of each NaPGA fraction, we plotted and determined, on a chromatogram, eluent volumes belonging to the chromatographic peak (V_{el}) and the complete volume of the gel-bed (V_t), which was identical with the positive peak originating from the Na-ions of the sample at the end on the chromatogram, and with the negative peak showing a lack of lithium on the chromatogram of the sample measured in the form of free acid, respectively (Figs 1 and 2).

On the chromatogram of NaPGA fractions calibrated by light scattering measurement method, we determined eluent volumes belonging to the peaks (V_{el}) and represented these figures, as the function of logarithm of the molecular mass ($\log \overline{M}_W$) (Table 2).

The GPC study of standard NaPGA samples was conducted by a micro-column method. The volumes belonging to the eluent peaks of the chromatogram (V_{el}) (Table 2) were plotted versus the logarithms of molecular masses (log $\overline{M}_W$) (Fig. 4), and finally regression calculation was made.

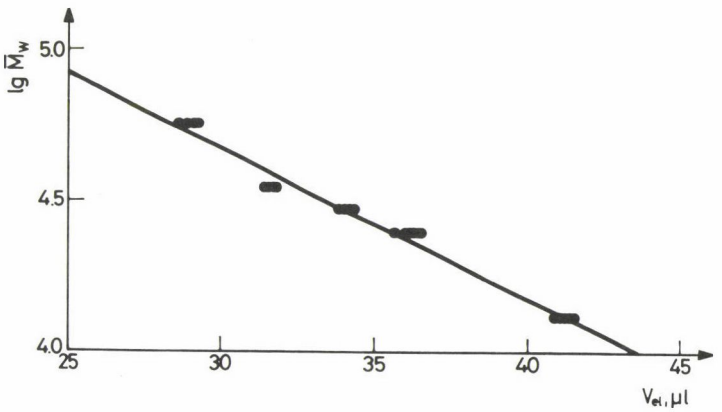

Fig. 4. Relationship between elution volume (V_{el}) and log molecular weight (log$\overline{M}_W$)

The line formed by points indicated on Fig. 4 was determined by regression calculation:

$$\log M_W = -0.0498\ V_{el} + 6.172; \qquad (r = 0.991)$$

Measuring points fit well into the line determined by regression calculation, therefore, it can be applied as a calibration curve to determine molecular masses in the GPC study of unknown PGA samples.

We conducted the GPC study of experimental PGA samples produced in this way, as described above (Fig. 2). Eluent volumes belonging to the chromatographic peak were determined, and with the application of log $\overline{M}_W$ versus V_{el} calibration curve (Fig. 4), we also determined the molecular mass belonging to the peak.

Both viscosimetric and GPC methods are suitable for the determination of laboratory and pilot-plant PGA samples. For pectins originating from different firms (Genu, Grindsted, Herbstreith), we conducted, according to pilot-plant conditions, alkaline hydrolysis (see Para. 1.2.). Parallel with this, viscosimetric and GPC studies of the obtained NaPGA samples were examined. Experimental results are summarized in Table 3.

Table 3

Results of viscosimetric and GPC measurements of NaGPA samples

PGA	$[\eta]$	$\bar{M}_W \times 10^{-4a}$	V_{el} (μl)	$\bar{M}_W \times 10^{-4b}$	$\bar{M}_W \times 10^{-4c}$	$\bar{M}_n \times 10^{-4c}$
Genu	185.53	4.025	31.120	4.182	3.740	3.205
Grindstedt	244.55	4.664	30.604	4.438	4.122	3.484
Herbstreith	122.96	3.231	32.704	3.270	3.323	2.749

[a]On the basis of viscosimetric measurement
[b]On the basis of GPC measurement (V_{el})
[c]On the basis of GPC signal heights

Values of molecular mass measured by viscosimetric and GPC methods are, within a limit of error, well identical.

3. Conclusions

For the determination of molecular mass of polygalacturonate anion, produced by alkaline hydrolysis of pectin, a relatively simple and quick viscosimetric method is suggested, on the basis of the Mark-Houwink equation:

$$[\eta] = 4.368 \times 10^{-7} \bar{M}^{1.8737}$$

Data obtained provide identical data with the results of GPC method.

*

We are grateful to dr. TENNIKOVA for supply of new sorbent GMA-gel.

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Acta Alimentaria, Vol. 21 (3–4), pp. 351 (1992)

BOOK REVIEW

Food processing by ultra high pressure twin-screw extrusion

I. HAYAKAWA (Ed.)

Technomic Publishing Company, Inc., Lancaster, Pennsylvania, USA, 1992. 153 pages

The paperback contains 13 independent articles, dealing with the subject indicated in the title of the book. These articles have been since the second half of the eighties. First author of 11 paper is Isao HAYAKAWA, and Yusaku FUJIO and Nobuyuki HAYASHI are first authors of two other publications. They and 11 co-authors represent the community publishing this selection.

In 7 publications the KEI-45 and KEI-45-15 models of the Company Kowa Kogyo, Ltd, the twin-screw extruder (corotative type) has been applied as main processing equipment. However, these differed from each other in the screw configurations, in the revolution number, in their application for heating and cooling, and possibly in the construction of the restriction discharge. Six articles contain research results achieved without the use of any extruder. Nevertheless, the results can be applied in the extrusion technics.

Extruded kinds of foods:

Soy beans with original fat contents (flour and granulate), soy protein, starch, bakery yeast, protein of animal origin. One of the works uses wheat-gluten and another amylolytic enzyme as test material.

Physical, physico-chemical and chemical procedures have been discussed in the studies: treatment period, residence time distribution and the analysis of mixing relations by the use of indicator (tracer) materials. Electron microscope, ultra high pressure equipment with built-in spectrophotometer, double refraction apparatus, food rheometer for the determination of strain energy, differential scanning calorimeter were also used.

Use of blade shear cell for texture measurement, application of an apparatus for Fourier transform infrared analysis, gel electrophoresis, etc. are studied in the articles.

Some of the results: The three-dimensional protein structure gets modified in soy bean for chains of linear arrangement when extruded; twin-screw extrusion combined with heat treatment (cooking) proved to be effective for the inactivation of those components of soy bean, which are active from physiological point of view.

Sugar ester as hydrophylic surfactant improved the processing parameters of soy bean extrusion and reduced the internal friction of starch.

Dead spaces in the extruder can be reduced effectively with the use of counter screws. The inactive alpha-amylase can be regenerated by the use of high pressure (150–200 MPa). The starch gelatinization was promoted by extrusion cooking even in case of low water contents (20%).

A detailed subject index and authors' index are serviceable parts of the book.

I. KÖRMENDY

Acta Alimentaria 21, 1992
Akadémiai Kiadó, Budapest

ANNOUNCEMENT

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- Quality assurance and control (safety, analytical methods and legal issues).

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RECENTLY ACCEPTED PAPERS

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Fermentation and properties of thermostable proteinase
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NOTICE TO CONTRIBUTORS

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Figures and tables. Illustrations must be cited and numbered in the order they appear in the text. All line drawings should be submitted as clear, glossy, black and white originals or photographs of good quality. The author's name and the title of the paper together with the serial number of the figure should be written on the back of each figure. Figures and Tables, each bearing a title, should be self-explanatory and numbered consecutively. The number of measurements (n), their mean values ($\bar{x}$) and standard deviations ($\pm s$) should be indicated. To prove the objectivity of conclusions drawn from the results mathematical statistical methods must be used. Quantitative evaluation of data is indispensable.

References. The reference list (Literature) should be in alphabetical order as follows:

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